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Systematic Exploration of the Antigen Binding Activity of Synthetic Peptides Isolated from the Variable Regions of Immunoglobulins*

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Sets of short (12 residues) cellulose-bound synthetic overlapping peptides derived from the sequences of the variable regions of the heavy and light chains of three different antibodies (an anti-thyroglobulin antibody, the HyHEL-5 anti-lysozyme antibody, and an anti-angiotensin II antibody) were used to systematically assess the antigen binding capacity of peptides from the antibody paratope outside their natural molecular context. Peptides enclosing one or several of the complementarity determining region (CDR) residues had antigen binding activity, although the most active peptides were not necessarily those bearing the greatest number of CDR residues. Several residues from the framework region, preceding or following the CDR, were found to play a role in binding. Affinity constants from 4.1×10^{-7} to 6.7×10^{-8} M^{-1} for the soluble form of 9 lysozyme-binding dodecapeptides were measured by BIAcore analysis. Alanine scanning of lysozyme-binding hexapeptides from the HyHEL-5 sequence identified 38 residues important for binding, of which 22 corresponded to residues that had been shown by x-ray crystallography to be at the interface between HyHEL-5 and lysozyme. Our results could be of interest for the rational identification of biologically active peptides derived from antibody sequences and in providing an experimental basis for mutagenesis of the antibody paratope.

Antibody molecules bind antigens with high affinity and specificity by synergistically using multiple noncovalent forces. The combining site (paratope), whose shape is complementary to the epitope on the antigen, is made up of the hypervariable regions, also called complementarity determining regions (CDRs)[1] (1). It is commonly accepted that there are three CDRs in the light chain (L1, L2, and L3) and in the heavy chain (H1, H2, and H3). These CDRs fold into turn structures that are stabilized by the β-sheet framework of the variable domains. The interface between antibodies and antigens has been precisely described by x-ray crystallographic studies, and several complexes between Fab fragments of monoclonal antibodies and peptide or protein antigens have been recently described (for reviews see Refs. 2–4). The structures of antibody-antigen complexes indicate that at least four of the CDRs, and in some cases all six CDRs, contribute to antigen binding (5). Residues in the framework have rarely been reported to participate in this interaction (6, 7).

Antibody-peptide or antibody-protein complexes are excellent model systems to study the physicochemical requirements for molecular recognition. Unfortunately, it is a difficult task to obtain crystals suitable for the structural elucidation of antibody fragments in complex with proteins or peptides. Therefore, other approaches to obtain information about the key residues involved in the interaction would be very useful, in particular for paratope mutagenesis. Some workers have demonstrated that synthetic peptides derived from the amino acid sequences of CDRs bind antigens with specificities similar to those of the original antibody molecules (8–15). Such peptides have very often been chosen in the CDR3 of the V_H sequence, which is considered to play a prominent role in defining antibody reactivity. However, the capacity of synthetic peptides derived from the variable regions of a given antibody to bind an antigen has never been probed in a systematic manner, *i.e.* in assessing the antigen binding capacity of every overlapping peptide from the V_H and V_L sequences.

In this study, we present the results obtained by measuring the ability of an antigen to bind to sets of immobilized overlapping peptides of uniform size covering the amino acid sequences of the V_H and V_L domains of three different antibodies. The peptides were prepared by the Spot method (16), which has previously been successfully used to identify peptide epitopes recognized by anti-protein antibodies and further developed to map protein-protein interaction sites (17). Our results indicate that numerous peptides show antigen binding capacity, most of them exhibiting measurable affinities in BIAcore, and that paratope residues important for antigen recognition can be identified by peptide analysis.

EXPERIMENTAL PROCEDURES

Antigens—Hen egg white lysozyme was from Sigma, and human thyroglobulin was from UCB-Pharma (Nanterre, France). Synthetic angiotensin II and an N-terminally biotinylated derivative were prepared as described below.

Protein Biotinylation—2 mg of the antigen in 2 ml of bicarbonate buffer (pH 8.6) were biotinylated by using a commercial reagent (Amersham RPN2202) according to the manufacturer's protocol. Biotinylated antigens were incubated with 0.1 M glycine (1 h, 37 °C) and then stored in phosphate-buffered saline at −20 °C.

Amino Acid Sequences of Antibodies—The numbering of the amino acid sequences of the variable regions was that of Wu and Kabat (1). The amino acid sequence of the anti-angiotensin II antibody 4D8 was established by sequencing the cDNAs corresponding to the heavy and light chains after reverse transcription of the mRNA from the 4D8 hybridoma (18). The amino acid sequences of the anti-lysozyme anti-

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[1]The abbreviations used are: CDR, complementarity determining region; V_H, variable region of the heavy chain; V_L, variable region of the light chain; Fmoc, *N*-(9-fluorenyl)methoxycarbonyl; HPLC, high pressure liquid chromatography.

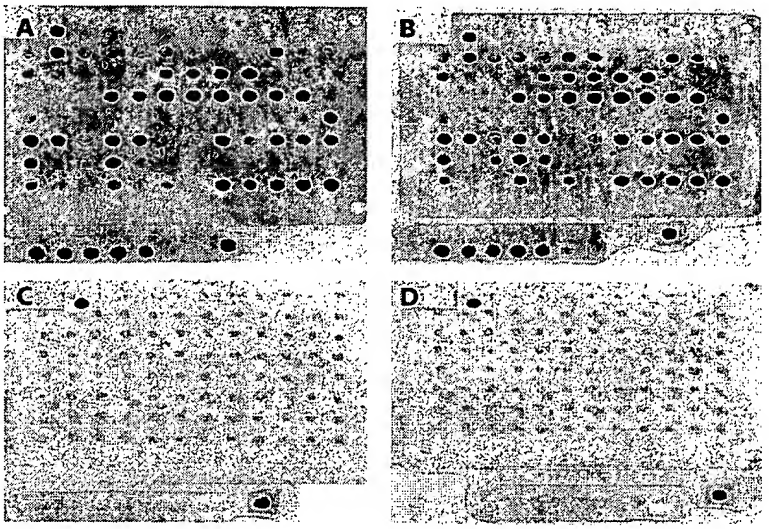

FIG. 1. **Reactivity of overlapping dodecapeptides derived from the sequence of the anti-lysozyme antibody HyHEL-5 with biotinylated lysozyme.** The membrane on which the peptides were synthesized was incubated with 1 μg/ml biotinylated lysozyme (*A*), 1 μg/ml biotinylated lysozyme in the presence of human normal serum diluted 1:50 (*B*), 1 μg/ml biotinylated lysozyme in the presence of 0.1 mg/ml nonbiotinylated lysozyme (*C*), or alkaline phosphatase-streptavidin (1:3000) (*D*).

body HyHEL-5 and the anti-human thyroglobulin Tg10 antibody were taken from the literature (19, 20).

Peptide Synthesis on Cellulose Membrane—The general protocol has been described previously (21). Membranes were obtained from Abimed (Langenfeld, Germany). Fmoc amino acids and *N*-hydroxybenzotriazole were from Novabiochem. An ASP222 robot (Abimed) was used for the coupling steps. All peptides were acetylated at their N terminus. After the peptide sequences had been assembled, the side-chain protecting groups were removed by trifluoroacetic acid treatment (16).

Immunoassay with Cellulose-bound Peptides—The general protocol was the same as for epitope analysis (16) except for the use of a biotinylated antigen (90-min incubation at 37 °C). After washing the membrane, a 1:3000 dilution of an alkaline phosphatase-streptavidin conjugate (Sigma) was incubated for 30 min at room temperature. Binding was revealed by addition of a phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate-3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma), giving a blue precipitate on those spots having bound the alkaline phosphatase-streptavidin conjugate.

A plot of spot intensities was obtained with the NIH Image software after scanning the membrane as described (21). Color intensities were calculated with reference to a black spot taken as the maximum of a 0–255 scale (arbitrary units). To allow the reuse of the membrane, it was sequentially treated by dimethylformamide, 6 M urea, and 10% acetic acid in ethanol so as to remove the precipitated dye and molecules bound to peptides. The reactivity of each antigen was assessed in two or three independent experiments.

Synthesis of Soluble Peptides—All soluble peptides were synthesized on an Abimed AMS 422 synthesizer by Fmoc chemistry. Except for angiotensin II, a spacer sequence (YKK) was added at the N terminus of every peptide followed by a biotin residue. Peptides were deprotected and released from the resin by trifluoroacetic acid treatment in the presence of appropriate scavengers. Peptides were lyophilized, and their purity was assessed by HPLC. When necessary, peptides were purified to greater than 90% HPLC homogeneity.

Real Time Analysis of Peptide-Antigen Interaction by BIAcore—The BIAcore apparatus was from BIAcore (Uppsala, Sweden). All experiments were carried out at 25 °C. N-terminally biotinylated peptides (5 μg/ml in Hepes-buffered saline buffer) were immobilized on a streptavidin-coated sensor chip. The injection was performed at a flow rate of 5 μl/min. The net surface plasmon resonance signal for immobilized peptides was found to be about 25–30 resonance units after completion of the chip regeneration cycle, which corresponds to 25–30 pg/mm^2 (12–15 fmol/mm^2). The binding kinetics of lysozyme to immobilized peptides was determined by injecting lysozyme (2–4 μM) in Hepes-buffered saline buffer (running buffer) at a flow rate of 10 μl/min. Dissociation was observed in running buffer without dissociating agents at a flow rate of 10 μl/min. The kinetic parameters of the binding reactions were determined using BIAevaluation 2.1 software (22). The dissociation rate (off-rate) constant k_d was determined from a plot of $\ln(R_0/R)$ *versus* time, R being the surface plasmon resonance signal at time t; the association rate constant (on-rate) k_a was determined from a plot of $\ln[\text{abs}(dR/dt)]$ *versus* time. The apparent equilibrium dissociation constant was calculated from the kinetic constants: $K_D = k_d/k_a$.

Analysis of Framework-CDR Interaction by Molecular Modeling—The coordinates of the HyHEL-5-lysozyme complex (3hfl; Ref. 23) were used with the Insight II software to identify CDR residues in contact (*i.e.* less than 3.4 Å apart) with amino acids from the framework that were found to be important by Spot peptide analysis.

RESULTS

Capacity of Peptides Derived from the V_H and V_L Sequences of Three Different Antibodies to Specifically Bind the Cognate Antigen—The capacity of short peptides derived from the variable regions of three different antibodies to bind the cognate antigen was investigated in a systematic manner. The V_H and V_L amino acid sequences were presented as sets of 110 overlapping dodecapeptides (2-residue frameshift) synthesized according to the Spot method (16, 21). In this method, the peptides remain attached to the cellulose membrane used for their synthesis, and their immunoreactivity is probed by incubating the membrane with a solution containing the ligand. As an example, Fig. 1 shows the results obtained with peptides derived from the variable regions of HyHEL-5, an anti-lysozyme antibody (6). Biotinylated lysozyme (1 μg/ml; 6×10^{-8} M) bound to several peptides derived from the V_H and V_L sequences of HyHEL-5 (Fig. 1*A*). A detailed analysis of this interaction is provided under "Relationships between the Sequence of Antigen-binding Peptides and CDR Location in the HyHel-5 Model." The binding pattern was not affected by incubating the biotinylated lysozyme in a 50-fold dilution of normal human serum, *i.e.* in the presence of a high concentration of proteins unrelated to lysozyme (Fig. 1*B*). However, when lysozyme (100 μg/ml) was added to the incubation mixture, binding no longer occurred (Fig. 1*C*). No binding was observed with the alkaline phosphatase-streptavidin complex (Fig. 1*D*) except on two control peptides that have the HPQ sequence recognized by streptavidin (24). The binding of lysozyme to immobilized peptides is therefore specific.

In the second model studied, biotinylated thyroglobulin (1 μg/ml; 3×10^{-9} M) was also found to bind several peptides from the V_H and V_L domains of the anti-thyroglobulin antibody Tg10 (25). The reactivity was observed in six regions of the membrane that broadly corresponded to peptides containing CDR residues (data not shown). The reactivity was abolished in the presence of an excess of thyroglobulin.

In another set of experiments, the V_H and V_L domains of the high affinity anti-angiotensin II antibody 4D8 ($K_D = 1.3 \times 10^{11}$ M; 18) were scanned by overlapping dodecapeptides. Biotinylated angiotensin II (1 μg/ml; 1×10^{-6} M) bound to peptides corresponding broadly to the three CDRs of V_L and to H1, whereas peptides corresponding to H2 and H3 showed less

TABLE I

Sequences of the dodecapeptides derived from the V_H and V_L of antibody HyHel-5 and their reactivity with biotinylated lysozyme

Framework residues are colored in black, and CDR residues are in red. Shaded areas correspond to peptides showing significant binding.

Peptide number	Peptide sequence	Color Intensity of the spots (±SD)
2	AMRHPQPCG CONTROL PEPTIDE	222(±9)
	CDR L1	
3	DIVLTQSPAIMS	32(±5)
4	VLTQSPAIMSAS	35(±2)
5	TQSPAIMSASPG	36(±4)
6	SPAIMSASPGEK	40(±3)
7	AIMSASPGEKVT	40(±3)
8	MSASPGEKVTMT	39(±1)
9	ASPGEKVTMTCS	35(±4)
10	PGEKVTMTCSAS	43(±4)
11	EKVTMTCSASSS	36(±6)
12	VTMTCSASSSVN	41(±4)
13	MTCSASSSVNYM	103(±5)
14	CSASSSVNYMYW	241(±9)
15	ASSSVNYMYWQ	126(±20)
16	SSVNYMYWQQK	128(±12)
17	VNYMYWQQKSG	105(±7)
18	YMYWQQKSGTS	145(±4)
19	YWQQKSGTSPK	168(±7)
20	YQQKSGTSPKRW	61(±9)
21	QKSGTSPKRWIY	47(±14)
	CDR L2	
22	SGTSPKRWIYDT	225(±5)
23	TSPKRWIYDTSK	171(±4)
24	PKRWIYDTSKLA	142(±14)
25	RWIYDTSKLAS	194(±5)
26	IYDTSKLASGVP	75(±4)
27	DTSKLASGVPVR	71(±8)
28	SKLASGVPVRFS	97(±2)
29	LASGVPVRFSGS	156(±3)
30	SGVPVRFSGSGS	207(±5)
31	VPVRFSGSGSGT	194(±11)
32	VRFSGSGSGTSY	225(±8)
33	FSGSGSGTSYSL	235(±10)
34	GSGSGTSYSLTI	47(±5)
35	GSGTSYSLTISS	52(±2)
	CDR L3	
36	GTSYSLTISSME	60(±5)
37	SYSLTISSMETE	56(±4)
38	SLTISSMETEDA	36(±3)
39	TISSMETEDAAE	36(±4)
40	SSMETEDAAEYY	227(±3)
41	METEDAAEYYCQ	200(±4)
42	TEDAAEYYCQQW	241(±2)
43	DAAEYYCQQWKR	252(±3)
44	AEYYCQQWKRNP	242(±8)
45	YYCQQWKRNPTF	244(±10)
46	CQQWKRNPTFGG	250(±3)
47	QWKRNPTFGGGT	244(±3)
48	KRNPTFGGGTKL	90(±5)
49	NPTFGGGTKLEI	104(±7)
50	PTFGGGTKLEIK	30(±10)
	CDR H1	
51	VQLQQSGAELMK	35(±5)
52	LQQSGAELMKPG	36(±3)
53	QSGAELMKPGAS	46(±3)
54	GAELMKPGASVK	48(±4)
55	ELMKPGASVKIS	43(±4)
56	MKPGASVKISCK	44(±3)
57	PGASVKISCKAS	49(±1)
58	ASVKISCKASGY	46(±3)
59	VKISCKASGYTF	121(±8)
60	ISCKASGYTFSD	235(±4)
61	CKASGYTFSDYW	253(±2)
62	ASGYTFSDYWIE	241(±2)
63	GYTFSDYWIEWV	96(±4)
64	TFSDYWIEWVKQ	235(±7)
65	SDYWIEWVKQRP	223(±10)
66	YWIEWVKQRPGH	62(±5)
67	IEWVKQRPGHGL	129(±5)
68	WVKQRPGHGLEW	211(±7)
	CDR H2	
66	YWIEWVKQRPGH	62(±5)
67	IEWVKQRPGHGL	129(±5)
68	WVKQRPGHGLEW	211(±9)
69	KQRPGHGLEWIG	181(±3)
70	RPGHGLEWIGEI	254(±1)
71	GHGLEWIGEILP	186(±7)
72	GLEWIGEILPGS	213(±4)
73	EWIGEILPGSGS	180(±35)
74	IGEILPGSGSTN	49(±2)
75	EILPGSGSTNYN	149(±10)
76	LPGSGSTNYNER	237(±2)
77	GSGSTNYNERFK	167(±2)
78	GSTNYNERFKGK	60(±5)
79	TNYNERFKGKAT	69(±2)
80	YNERFKGKATFT	82(±6)
81	ERFKGKATFTAD	62(±2)
82	FKGKATFTADTS	54(±2)
83	GKATFTADTSSS	49(±1)
84	ATFTADTSSSTA	44(±2)
85	FTADTSSSTAYM	130(±14)

intense reactivity (not shown). When biotinylated angiotensin II (1 μg/ml) was incubated with 1 mg/ml angiotensin II, the signal markedly decreased. No cross-reactivity was observed

TABLE I—*continued*

	CDR H3	
86	ADTSSSTAYMQL	74(±2)
87	TSSDTAYMQLSS	77(±3)
88	SDTAYMQLSSLT	165(±10)
89	TAYMQLSSLTSE	106(±2)
90	YMQLSSLTSEDS	175(±5)
91	QLSSLTSEDSGV	48(±3)
92	SSLTSEDSGVYY	235(±5)
93	LTSEDSGVYYCL	202(±5)
94	SEDSGVYYCLSG	236(±7)
95	DSGVYYCLSGNY	238(±3)
96	GVYYCLSGNYDF	207(±4)
97	YYCLSGNYDFDG	234(±1)
98	CLSGNYDFDGWG	256(±1)
99	LSGNYDFDGWGQ	252(±2)
100	SGNYDFDGWGQG	246(±1)
101	GNYDFDGWGQGT	249(±1)
102	NYDFDGWGQGTT	112(±9)
103	YDFDGWGQGTTL	96(±5)
104	AMRHPQPCG CONTROL PEPTIDE	225(±5)

with any of the three antigens on peptides derived from unrelated antibodies. All of these results showed that 12-mer peptides derived from the amino acid sequence of the V_H and V_L domains of three different antibodies are capable of being specifically recognized by their cognate antigen.

Relationships between the Sequence of Antigen-binding Peptides and CDR Location in the HyHEL-5 Model—Table I shows the results of the quantitative analysis of the binding of biotinylated lysozyme to cellulose-bound peptides derived from the amino acid sequence of HyHEL-5. The majority of peptides containing only framework residues displayed no binding activity (peptides 3–8, 20–21, 34–39, 51–58, 84, and 86–87). However, several peptides (31–33, 40, 59, 68–69, 85, 88–90, and 92–93) that also contained only framework residues were reactive. The possible molecular basis for this reactivity is discussed under "Precise Identification of Residues Contributing to Antigen Binding and Camparison with Contact Residues Defined by X-ray Crystallography". Analysis of the relationships between the amino acid sequence and the binding properties (Table I) indicated that strong binding capacity was detectable when certain CDR residues were present in the peptide sequence. For example, the sequence VTMTCSASSSVN from L1 (peptide 12) had no activity, but the following (overlapping) sequence MTCSASSSVNYM (peptide 13) possessed activity; therefore, the motif YM clearly contributed to binding. Other residues from CDRs were thus identified as contributors: DT from L2 at peptide 22, SD from H1 at peptide 60, and YH from H2 at peptide 75. Some residues not belonging to CDRs also apparently contributed to binding activity: GS at peptide 29, YY at peptide 40, TF at peptide 59, KQ at peptide 64, GL at peptide 67, LT at peptide 88, and YY at peptide 92. Also, a decrease in the binding capacity of certain peptides occurred when residues from the CDRs disappeared from the peptide sequence, *e.g.* the difference in the activities of peptides 19 and 20 could be attributed to the absence of YW in peptide 20. The following CDR residues were thus identified: QW from L3 (difference in binding to peptide 47 *versus* 48), SD from H1 (peptide 65 *versus* 66), GS from H2 (peptide 77 *versus* 78), and DF from H3 (peptide 101 *versus* 102). When certain residues not belonging to the CDR were removed from the amino acid sequence of a binding peptide, the antigen binding capacity was reduced: absence of RW in the sequence of peptide 26, of FS in peptide 34, of AS in peptide 63, of EW in peptide 74, and of YM in peptide 91. Therefore, it seems that the binding of antigen to cellulose-bound peptides is based on the presence in their sequence of certain residues from the CDRs and in several instances of certain framework residues neighboring the CDRs.

Precise Identification of Residues Contributing to Antigen Binding and Comparison with Contact Residues Defined by X-ray Crystallography—Alanine scanning of hexapeptides derived from each previously identified binding sequence from

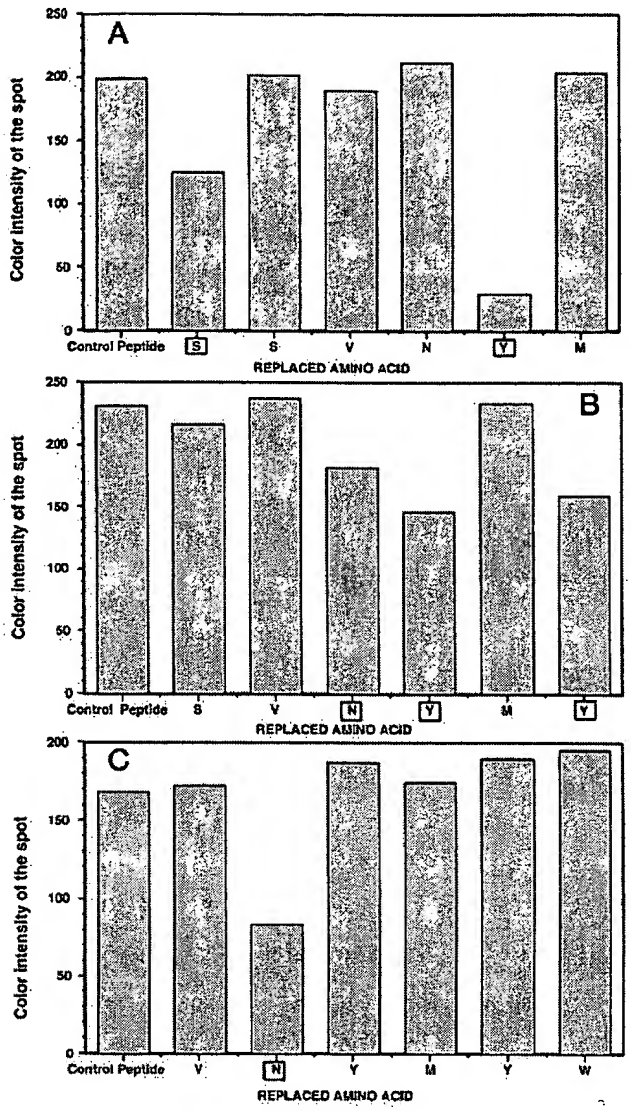

FIG. 2. **Determination of residues contributing to the binding of biotinylated lysozyme to sets of alanine analogs of hexapeptides derived from the L1 sequence of HyHEL-5 antibody.** Each *bar* represents the reactivity of the hexapeptide whose sequence comprises an Ala residue in place of the indicated amino acid. *Boxed letters* indicate amino acids considered as contributors. *A*, alanine scanning of peptide SSVNYM. *B*, alanine scanning of peptide SVNYMY. *C*, alanine scanning of peptide VNYMYW.

the V_H and V_L domains of HyHEL-5 was performed to identify the exact residues contributing to antigen binding. The study of the CDR L1 region is given as a detailed example (Fig. 2; see legend of Fig. 3 for the amino acid numbering of the V_H and V_L sequences). Three hexapeptides (SSVNYM, SVNYMY, and VNYMYW) and each of their six alanine analogs were synthesized by the Spot method and tested for reactivity with biotinylated lysozyme. Replacing Ser^{27} of peptide SSVNYM by an alanine residue led to a fall in the antigen binding capacity, whereas alanine replacement of Tyr^{32} led to disparition of the binding. Changing any of the other amino acids of the peptide SSVNYM did not modify the binding (Fig. 2*A*). For peptide SVNYMY, the three amino acids Asn^{31}, Tyr^{32}, and Tyr^{34} appeared to be important (Fig. 2*B*). Alanine scanning of peptide VNYMYW indicated that only Asn^{31} is important (Fig. 2*C*). In preliminary experiments, we noted that the contribution of a given amino acid to binding was not always the same when the surrounding sequence varied, probably reflecting conformational effects. Indeed, Tyr^{32} appeared to be a contributor in peptides SSVNYM and SVNYMY but not a contributor in peptide VNYMYW; also, the influence of Asn^{31} was apparently more critical in sequence VNYMYW than in other sequences. In fact, several hexapeptides had to be analyzed to define the residues contributing to the binding.

The contributing motif for the L1 region was therefore determined to be ^{27}S--NY-Y^{34} (Fig. 3), with all four residues belonging to the CDR. Using the same alanine-scanning approach, contributors were determined for each CDR region. For the L2 region, the motif was identified as ^{46}RWIYD^{50} (Fig. 3); one residue (Asp^{50}) belonged to the CDR, and four residues were from the framework sequence preceding L2. For the L3 region, the contributing motif was ^{91}WGR-P-F^{98} (Fig. 3). Residues Trp, Gly, Arg, and Pro were part of the CDR, Phe^{98} being in the framework. For the H1 region, the contributing motif ^{27}Y-FSDYW-EW^{36} comprised four residues (DYW-E) from the CDR and four residues from the framework. For the H2 region, the motif comprised a framework residue, Trp^{47}, and six residues in the middle of the CDR (^{54}S-S-NYHE^{61}). For the H3 region, the motif ^{91}YC--HGNYDF-W^{103} had five amino acids from the CDR (GNYDF) and four residues from the framework (YC--H and Trp, respectively on the N- and C-terminal sides of the CDR). The determination of critical residues for binding the antigen provided a possible explanation for the reactivity of certain framework sequences; for example, peptides 29–34 (Table I) contain an SGS sequence that is part of the CDR H2; peptides 68 and 69, could be reactive because of the presence of the LEW sequence very similar to the IEW motif of H1. The reactivity of peptide 88 could be due to a strong sequence similarity with peptides 16–18 from the L1 region (SSTAYM as compared with SSVNYM). Thus, a cross-reactivity phenomenon may explain some of the reactivities in framework peptides; however, no similar explanation for the reactivity of peptides 40, 59, 89–90, and 92–93 was found.

The residues identified here as important for the binding of lysozyme to peptides derived from the sequence of the V_H and V_L domains of HyHEL-5 were then compared with the residues involved in antigen binding in the crystal structure of HyHEL-5 Fab-lysozyme (6, 23) (Fig. 3). For the L1 region, three amino acids (NY-Y) identified by peptide analysis are implicated in the crystallographic antigen-antibody interaction; Ser^{27} was found to be a contributor in our analysis only. For the L2 region, only one CDR amino acid (Asp^{50}) was implicated by crystallography in the antigen-antibody complex. Alanine scanning of reactive peptides from this region pointed out the role of both Asp^{50} and of the stretch of four residues preceding it. For the L3 region, the ^{91}WGR-P^{96} residues of the antigen-antibody complex were identified by peptide analysis with, however, the implication of an additional residue (Phe^{98}). For the H1 region, four CDR residues (DYW-E) and one framework residue (Ser^{30}) were found by crystallography to be implicated in antigen recognition; by alanine scanning of hexapeptides, the same five amino acids, ^{30}SDYW-E^{35}, were found to be contributors. However, three other amino acids (Y-F------W) outside the CDR seemed to be implicated in the interaction of the peptide with the antigen. For the H2 region, which is a 17-residue long CDR, seven amino acids from the CDR and one in the framework play a role in the antigen-antibody interaction as determined by x-ray crystallography. By using the Spot method, the importance of three amino acids of the CDR (Ser^{54}, Ser^{56}, and Asn^{58}) and of the Trp^{47} residue of the framework was determined. Whereas the crystallographic results implicate the N-terminal part and the middle of the CDR, the C-terminal part of the CDR (motif YHE) seemed to play an important role when peptides

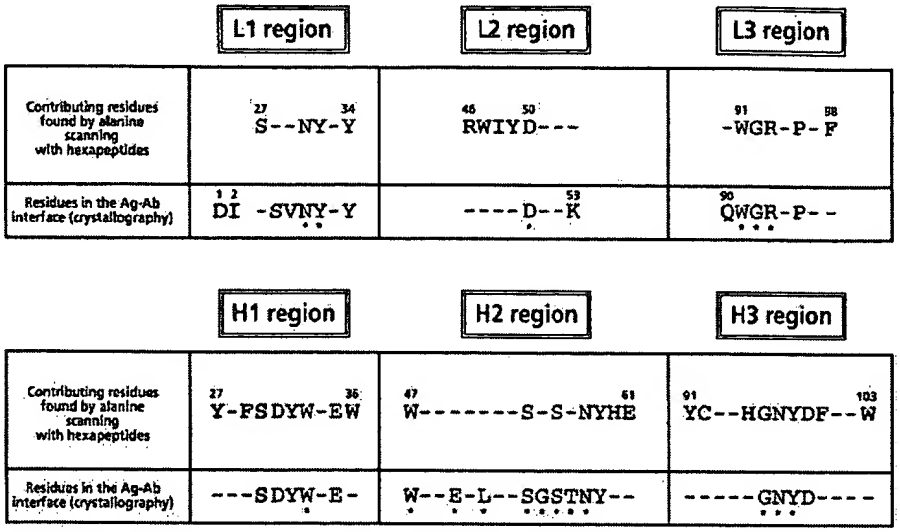

FIG. 3. **Comparison of residues found to contribute to the binding of lysozyme to peptides with residues involved in the paratope-epitope interface in the HyHEL-5 lysozyme complex (23).** CDR residues are colored in *red*. Residues labeled with *asterisks* correspond to residues in direct contact with lysozyme in the crystal structure. A residue was considered to be a contributor if the binding signal was reduced by at least 20% when it was replaced by an alanine. *Ab*, antibody. The complete amino acid sequences of the V_H and V_L of HyHEL-5 are given below. CDR residues are underlined.

VL

1 5 10 15 20 25 29 35 40 45 50 55 60 70 75 80 85 90 94 100 105

DIVLTQSPAIMSASPGEKVTMTCSASSSVNYMYWYQQKSGTSPKRWIYDTSKLASGVPVRFSGSGSGTSYSLTISSMETEDAAEYYCQQWGRNPTFGGGTKLEIK

VH

1 5 10 15 20 25 30 35 40 45 50 52a 55 60 65 70 75 80 82abc 85 90 95

ZVQLQQSGAELMKPGASVKISCKASGYTFSDYWIEWVKQRPGHGLEWIGEI L PGSGSTNYHERFKGKATFTADTSSSTAYM Q LNSLTSEDSGVYYCLH-

99 105 110

GNYDFDGWGQGTTLTVSS

SEQUENCES 1 AND 2

were used. For the H3 region, the motif GNYD found by crystallography is similar to the motif found by the alanine scanning of hexapeptides. However, five amino acids, which were defined by spot peptide analysis as contributors, were not implicated in the HyHEL-5-lysozyme interaction as defined in the crystal structure. Of these five contributors, four of them belonged to the framework (^{91}YC--H^{95}, N-terminal to the CDR, and Trp^{103}, C-terminal to the CDR).

The comparison between residues (totaling 38) important for lysozyme binding to V_H- and V_L-derived peptides and residues involved in HyHEL-5-lysozyme interaction permitted us to identify 14 of the 18 residues in direct crystallographic contact with the antigen (78%) and 22 of the 32 (69%) residues that are either in contact or partially buried at the antigen-antibody interface (23) (Fig. 3). Sixteen residues that were found to be important in the binding of biotinylated lysozyme to peptides did not correspond to amino acids at the antigen-antibody interface. The majority of them (12 of 16) were framework residues. Table II shows that all five contributors from the V_L framework and six of nine important residues from the V_H framework belong to the subset of framework residues from the "Vernier" zone, *i.e.* defined as residues that may adjust CDR structure and fine tune the fitting to antigen (26). By using the atomic coordinates of the HyHel-5-lysozyme complex, it was found that, except for Tyr^{91H} and Cys^{92H} (not in the Vernier zone), all the other framework residues identified as contributors to antigen binding by peptide analysis make contacts in the crystal structure with at least adjacent residues from the CDR (Table II). Therefore, this subset of residues could contribute by giving the peptide an appropriate conformation for antigen recognition.

TABLE II
Characterization of framework residues found to be important for antigen binding by peptide analysis

Contributing residue from the framework	Belonging to the Vernier zone[a]	Contact residue from the adjacent CDR (CDR)[b]
Arg^{46L}	Yes	Ala^{55L} (CDR L2)
Trp^{47L}	Yes	Leu^{54L}, Ala^{55L} (CDR L2)
Ile^{48L}	Yes	Thr^{51L}, Ser^{52L}, Lys53L (CDR L2)
Tyr^{49L}	Yes	Asp^{50L}, Lys^{53L}, Leu^{54L}, Ala^{55L} (CDR L2)
Phe^{98L}	Yes	Thr^{97L}, Gln^{89L}, Pro^{95L} (CDR L3)
Tyr^{27H}	Yes	Tyr^{32H} (CDR H1)
Phe^{29H}	Yes	Asp^{31H}, Try^{32H} (CDR H1)
Ser^{30H}	Yes	Asp^{31H} (CDR H1)
Trp^{36H}		Ile^{34H}, Glu^{35H} (CDR H1)
Trp^{47H}	Yes	Glu^{50H}, His^{60H} (CDR H2)
Try^{91H}		
Cys^{92H}		
His^{94H}	Yes	G95H, N96H, F100H, D101H (CDR H3)
Trp^{103H}	Yes	D101H, G102H (CDR H3)

[a] Ref. 26.
[b] Contact as defined under "Experimental Procedures." The list of contacts is not exhaustive.

Affinity Determination of the Interaction between Peptides and Lysozyme by BIAcore Analysis—Based on the sequences of the peptides used in the Spot assay, a series of peptides derived from the variable regions of HyHEL-5 was synthesized by conventional solid phase synthesis and used in BIAcore real time interaction analysis (27). Fig. 4*A* shows a typical sensorgram of the interaction of an immobilized biotinylated peptide (Lyso 1) with lysozyme; the fitting of the experimental associ-

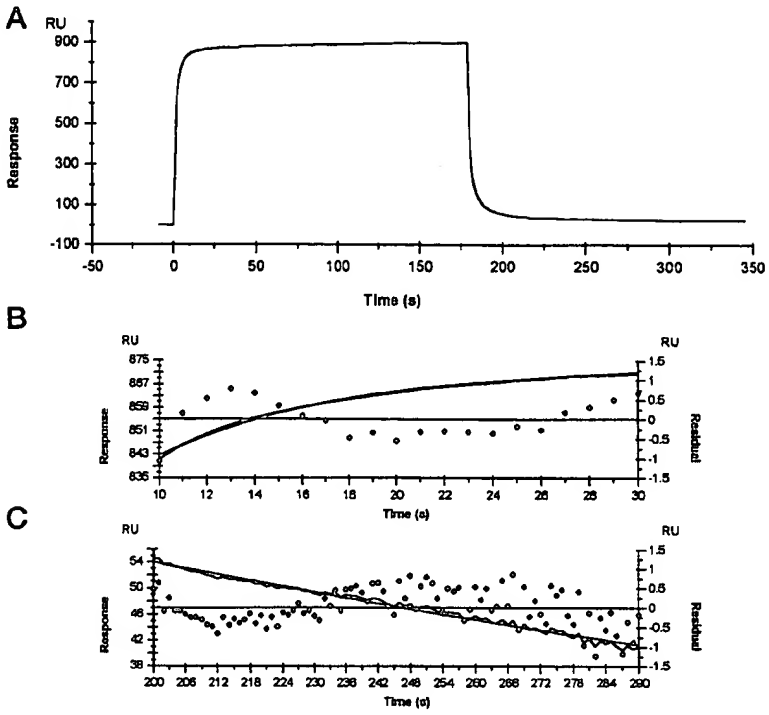

FIG. 4. **Surface plasmon resonance analysis of interaction between lysozyme and peptide Lyso 1.** *A*, sensorgram for the binding of lysozyme to peptide Lyso 1. *B*, on-rate constant determination using a one-site model with data from *A*; the experimental and theoretical curves and the residuals between these two curves are overlaid. *C*, off-rate constant determination using a one-site model with data from *A*; the experimental and theoretical curves and the residuals between these two curves are overlaid. *RU*, resonance units.

ation curve (Fig. 4*B*) and dissociation curve (Fig. 4*C*) with the theoretical curves indicated that a one-site interaction model satisfactorily described the binding. The same observations were made for all other measurable peptide interactions (not shown). Table III summarizes the results of the BIAcore study in which the kinetic parameters k_a and k_d were measured. Among 10 soluble peptides corresponding to sequences highly reactive in the Spot assay, at least one peptide from each of the six hypervariable regions of the HyHel-5 antibody was found to exhibit measurable binding with a K_D value in the $0.6-4 \times 10^{-7}$ M range (upper part of Table III). Two other soluble synthetic peptides (Lyso 15 and Lyso 17) showed no measurable binding in BIAcore analysis. For Lyso 6, Lyso 10, and Lyso 16, corresponding to peptides from the framework of HyHEL-5 exhibiting binding activity in Spot, only Lyso 6 displayed an affinity constant that was similar to that measured for the other peptides. As expected, irrelevant peptides or peptides derived from sequences of faintly or noncolored spots gave no binding in the BIAcore assay (bottom part of Table III). These results indicate that several short (12 residues), linear peptides identified on the basis of their reactivity in the Spot format gave rise to interaction of sufficient energy with lysozyme to be analyzed by real time interaction analysis. However, there were some peptides reactive in the Spot assay that did not show measurable affinity, making it difficult to infer BIAcore behavior from the results of the Spot analysis.

DISCUSSION

Antigen binding occurs through molecular contacts with several of the spatially juxtaposed CDRs of the V_H and V_L domains of the antibody molecule (28, 29). A systematic evaluation of the capacity of every peptide from the V_H and V_L regions to bind the antigen was performed in this study to assess the effect of keeping the sequence information but disrupting the precise molecular arrangement of the paratope. It was found that in the case of three different antigens (angiotensin II, hen egg white lysozyme, and human thyroglobulin), differing considerably in their size, numerous immobilized peptides from the V_H and V_L regions of the cognate antibody bind the antigen in a specific manner. Moreover, we have unpublished results showing that in the case of two other anti-protein antibodies the same observations could be made, arguing in favor of the generality of the phenomenon for anti-protein antibodies. Our present analysis indicates that peptides with antigen binding activity have one or several residues from the CDR in their amino acid sequence. Synthetic peptides enclosing complete CDRs generally displayed strong binding activity; however, peptides representing incomplete CDRs but including amino acids from sequences flanking the CDRs were also active, indicating contribution to the binding of some residues outside the CDR itself. It is not clear whether these residues contribute by directly contacting the antigen or by giving the reactive conformation to the peptide. We observed, however, that these residues often belong to the subset of framework residues that could modulate the conformation of the adjacent CDR (19, 26). It is therefore possible that the conformational state of reactive peptides immobilized on the cellulose membrane is very close to the conformation of the same sequence in the paratope. If this is the case, modification of a peptide residue critical for conformation would affect antigen binding in the same way that mutation of the corresponding framework residue would affect binding by altering CDR conformation. It was observed that some peptides including only framework residues do specifically bind to the antigen; this is possibly due in certain cases to occurrence in the framework sequence of similarities with the motifs contributing to antigen binding. It has been shown that a sequence homology of three residues in a peptide is sufficient to give rise to antigenic cross-reactivity (30). However, in several instances there is no obvious homology so that the chemical or structural basis for this reactivity remains to be assessed.

Binding of nine of thirteen synthetic peptides corresponding to those active in the Spot assay was observed by BIAcore real time interaction analysis. The dissociation constants of the interaction between soluble peptides and lysozyme in the 70–400 nM range are only 2 or 3 orders of magnitude higher than the K_D of the reaction between the whole antibody and ly-

TABLE III
Determination of the binding kinetics of the interaction between lysozyme and biotinylated peptides derived from the V_H and V_L sequences of HyHel-5

Name of the peptide	Peptide sequence	Color intensity[a]	Corresponding antibody region	k_a	k_d	K_D
				$10^4\ s^{-1}\ M^{-1}$	$10^{-3}\ s^{-1}$	$10^{-7}\ M$
Lyso 1	Biot-Sp-CSASSSVNYMYW	192	CDR L1	3.22	3.84	1.19
Lyso 3	Biot-Sp-SGTSPKRWIYDT	201	CDR L2	5.78	3.89	0.673
Lyso 4	Biot-Sp-RWIYDTSKLASG	171	CDR L2	2.38	6.27	0.857
Lyso 7	Biot-Sp-YYCQQWGRNPTF	180	CDR L3	2.33	3.37	1.45
Lyso 8	Biot-Sp-CKASGYTFSDYW	197	CDR H1	1.10	4.54	4.12
Lyso 9	Biot-Sp-TFSDYWIEWVKQ	191	CDR H1	1.33	3.70	2.78
Lyso 11	Biot-Sp-RPGHGLEWIGEI	203	CDR H2	1.99	3.19	1.60
Lyso 18	Biot-Sp-CLHGNYDFDGWG	254	CDR H3	2.69	3.67	1.36
Lyso 15	Biot-Sp-LPGSGSTNYHER	195	CDR H2	NM[b]	NM	NM
Lyso 17	Biot-Sp-GVYYCLHGNYDF	203	CDR H3	NM	NM	NM
Lyso 6	Biot-Sp-SSMETEDAAEYY	209	FRW	4.85	7.39	1.52
Lyso 10	Biot-Sp-WVKQRPGHGLEW	182	FRW	NM	NM	NM
Lyso 16	Biot-Sp-NSLTSEDSGVYY	220	FRW	NM	NM	NM
Lyso 2	Biot-Sp-SSVNYMYWYQQK	84	CDR L1	NM	NM	NM
Lyso 5	Biot-Sp-IYDTSKLASGVP	35	CDR L2	NM	NM	NM
Lyso 12	Biot-Sp-EWIGEILPGSGS	57	CDR H2	NM	NM	NM
Lyso 13	Biot-Sp-IGEILPGSGSTN	40	CDR H2	NM	NM	NM
Lyso 14	Biot-Sp-EILPGSGSTNYH	77	CDR H2	NM	NM	NM
Lyso 19	Biot-Sp-LQQSGAELMKPG	32	FRW	NM	NM	NM
Lyso 20	Biot-Sp-GKATFTADTSSS	35	FRW	NM	NM	NM
	Biot-Sp-AngiotensinII	ND	IRREL.	NM	NM	NM
	Biot-Sp-GAD-peptide	ND	IRREL.	NM	NM	NM

[a] The color intensities of the spots were measured after incubation of the membrane with biotinylated lysosyme at 0.1 μg/ml. ND, not determined.
[b] NM, not measurable.

sozyme (31). The kinetics of binding were characterized by rapid dissociation rates, probably due to a small number of interactions between the peptide and the antigen. To improve the binding capacity of such peptides derived from antibody sequences, it is suggested that cyclization could be useful in locking an active conformer (14, 32). Four peptides that were active in the Spot assay did not give measurable affinities in the BIAcore analysis, indicating that detection of antigen binding could be facilitated by the high density of peptides at the surface of the paper (about 3–11 nmol/mm^2; Ref. 16), which is not the case in the BIAcore format where the peptide density is as low as 10 fmol/mm^2.

The identification of short amino acid sequences recognized by peptide/protein antigens may have important applications for the screening of bioactive peptides based upon antibody sequences (11) in the current trend to redefine the minimal antigen-binding fragment (33) with the intention of preparing miniantibodies (34). Our systematic approach provides a way to select, on an experimental basis, peptide sequences with antigen binding activity. The results indicate that such sequences do not always correspond to peptides containing only CDR residues; in fact, residues from the framework were here found to play a critical role in the activity of CDR-based peptides, extending previous observations (13). Several residues that were found to contribute to peptide-lysozyme interaction were not residues located at the paratope-epitope interface defined by x-ray crystallography. They were mainly aromatic residues, suggesting that this type of residue plays a particular role in the efficiency of the interaction mechanisms. The role of tyrosine or phenylalanine residues in increasing the binding properties of cyclic peptides based on a CDR-like sequence of the CD4 molecule has been noticed (14, 15).

The identification of residues contributing to the binding of the antigen to peptides was performed in the HyHEL-5 antibody model for which detailed three-dimensional information on the interface between the antibody paratope and the antigenic protein is available (6, 23). Our identification was made in a blind manner and further compared with the results of x-ray crystallography. 22 of the residues determined as important in peptides for binding biotinylated lysozyme belonged to the set of 32 residues in contact with the antigen or buried in the paratope-epitope interface (6, 23), indicating that the binding that was observed with short peptides was consistent with the binding of the antigen to the whole antibody. The capacity of the method we used here to pinpoint residues potentially involved in the antigen-antibody interface could be valuable for the design of experiments aimed at mutating antibody-binding sites; in this context, not knowing exactly whether residues identified as important play a structural or functional role would not be detrimental. We suggest that peptide analysis conjugated with powerful binding site topography prediction methods (35, 36) could be a valuable strategy for antibody engineering.

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Synthetic Peptides Derived from the Variable Regions of an Anti-CD4 Monoclonal Antibody Bind to CD4 and Inhibit HIV-1 Promoter Activation in Virus-infected Cells*

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The monoclonal antibody (mAb) ST40, specific for the immunoglobulin complementarity-determining region (CDR) 3-like loop in domain 1 of the CD4 molecule, inhibits human immunodeficiency virus type 1 (HIV-1) promoter activity and viral transcription in HIV-infected cells. To design synthetic peptides from the ST40 paratope that could mimic these biological properties, a set of 220 overlapping 12-mer peptides frameshifted by one residue, corresponding to the deduced ST40 amino acid sequence, was synthesized by the Spot method and tested for binding to recombinant soluble CD4 antigen. Several peptides that included in their sequences amino acids from the CDRs of the antibody and framework residues flanking the CDRs were found to bind soluble CD4. Eleven paratope-derived peptides (termed CM1–CM11) were synthesized in a cyclic and soluble form. All the synthetic peptides showed CD4 binding capacity with affinities ranging from 1.6 to 86.4 nM. Moreover, peptides CM2, CM6, CM7, CM9, and CM11 were able to bind a cyclic peptide corresponding to the CDR3-like loop in domain 1 of CD4 (amino acids 81–92 of CD4). Peptide CM9 from the light chain variable region of mAb ST40 and, to a lesser extent, peptides CM2 and CM11 were able to inhibit HIV-1 promoter long terminal repeat-driven β-galactosidase gene expression in the HeLa P4 HIV-1 long terminal repeat β-galactosidase indicator cell line infected with HIV-1. The binding of mAb ST40 to CD4 was also efficiently displaced by peptides CM2, CM9, and CM11. Our results indicate that the information gained from a systematic exploration of the antigen binding capacity of synthetic peptides from immunoglobulin variable sequences can lead to the identification of bioactive paratope-derived peptides of potential pharmacological interest.

The CD4 molecule is a transmembrane glycoprotein (58 kDa) found on thymocytes, mature T-cells, macrophages, monocytes, and Langerhans' cells (1). This surface protein is required to shape the T-cell repertoire during thymic development (2) and to permit appropriate activation of mature T-cells through adhesion with class II major histocompatibility complex molecules and the T-cell receptor (3). Engaged CD4 subsequently plays a role in signal transduction by association with the protein-tyrosine kinase $p56^{lck}$ (4). Besides its physiological function, the CD4 surface glycoprotein, in association with chemokine receptors, acts as a receptor for HIV-1 [1] entry into cells (5–7). CD4 is a member of the immunoglobulin gene superfamily and consists of four extracellular domains (D1–D4) showing structural homology to immunoglobulin variable regions, a membrane-spanning region, and a cytoplasmic tail (8); in D1, there are three CDR-like regions (9, 10). The CDR2-like loop of D1 has been identified as the primary binding site for the HIV envelope glycoprotein gp120 (11–13), whereas the CDR3-like region represents a CD4 target for inhibition of the class II major histocompatibility complex-restricted immune responses (14–18) and HIV replication (19–24). Previous studies have shown that CDR3-like peptide analogs are strong inhibitors of these functions (14, 16–18, 25–28), probably interfering with CD4 dimerization (29, 30). Similarly, mAbs such as ST40 that bind to the CDR3-like loop in D1 of CD4 inhibit HIV-1 replication in infected cells at a post CD4/gp120 binding step (24).

Antibody paratopes result from the interactions between immunoglobulin variable heavy (V_H) and light (V_L) chains. The diversity of paratopes is mainly generated by the sequences of the CDRs found in V_H and V_L, which are exposed hypervariable loop structures. Antigen binding by peptide sequences from selected CDRs of mAbs has been demonstrated to have specificities similar to those of the original antibody molecule (31–40). Our previous results showed that the systematic exploration of the antigen binding capacity of short peptides derived from an antibody sequence leads to the identification of numerous paratope-derived peptides (PDPs) that display significant affinity for the antigen (40). Therefore, this approach could be useful to identify potentially biologically active peptides from the sequence of a pharmacologically active antibody.

In this study, we have established the nucleotide sequences

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ005354 and AJ005355.

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[1] The abbreviations used are: HIV-1, human immunodeficiency virus type 1; D1, domain 1; CDR, complementarity-determining region; mAb, monoclonal antibody; V_H, variable region of the heavy chain; V_L, variable region of the light chain; PDP, paratope-derived peptide; sCD4, soluble CD4; PBS, phosphate-buffered saline; Fmoc, *N*-(9-fluorenyl)methoxycarbonyl; HPLC, high pressure liquid chromatography; LTR, long terminal repeat.

of the V_H and V_L regions of mAb ST40. A set of immobilized overlapping dodecapeptides covering the deduced amino acid sequences of mAb ST40 variable regions was prepared by the Spot method (40, 41). The ability of biotinylated soluble CD4 (sCD4) to bind these peptides was then investigated and led to the selection of peptides with CD4 binding activity. All the selected PDPs prepared in a soluble cyclic form showed CD4 binding capacity, and three of them blocked HIV-1 promoter activity and efficiently competed with mAb ST40 for binding to CD4.

EXPERIMENTAL PROCEDURES

Soluble CD4—Recombinant purified sCD4, kindly provided by Professor D. Klatzmann (Hopital de La Pitié, Paris), comprised the four external domains of CD4 (42). sCD4 (280 μg in 600 μl of bicarbonate buffer, pH 8.6) was biotinylated using a commercial reagent (Amersham Pharmacia Biotech RPN2202) according to the manufacturer's instructions. Biotinylated sCD4 was stored in PBS at −20 °C until use.

Cloning of mAb ST40 V_H and V_L Genes—The murine hybridoma cell line that produces mAb ST40/F142-63 (IgG1,κ) was a kind gift from Dr. D. Carrière (Sanofi Recherche, Montpellier, France) (43). Total RNA was extracted from 3×10^6 hybridoma cells using the TRIzol™ technique (Life Technologies Inc., Paisley, United Kingdom). The V_L gene of the ST40 antibody was obtained by polymerase chain reaction amplification. Briefly, reverse transcription was performed with 2 μg of total RNA, the reverse transcriptase Superscript (Life Technologies Inc.), and the primer OPP-SoCκ3′ (5′-CGCGCAGATCTAACACTCATTCCT-GTTGAAGC-3′), which contains the reverse complement of codons 208–214 of Cκ. One μl of first strand cDNA was used as matrix for the polymerase chain reaction to amplify the ST40 V_L/C_L genes using Vent DNA polymerase (New England Biolabs, Hitchin, UK) and the primers OPP-SoCκ3′ and OPP-SoVκ5′ (5′-GA(C/T/A)ATTGAGCTCAC(C/A)CA-G(T/A)CTCCA-3′). These primers contained restriction sites (underlined) for cloning. The degenerate primer OPP-SoVκ5′ was chosen as the consensus sequence of codons 5–8 in murine FR1 Vκ. The polymerase chain reaction-amplified DNA product was digested sequentially with *Bgl*II and *Sac*I (New England Biolabs) and purified on a 1.5% low-melting temperature agarose gel (Life Technologies Inc.). This digested DNA was ligated to pUC19 that was prepared in a similar manner. The V_L cDNA sequence was determined by double-stranded sequencing using the dideoxy chain termination method with the T7 sequencing kit (Amersham Pharmacia Biotech, Uppsala). The V_H gene of the ST40 antibody was isolated from a cDNA library. Briefly, poly(A)$^+$ RNAs were magnetically separated from total RNAs by hybridization with a biotinylated oligo(dT) primer and then captured by streptavidin coupled to paramagnetic beads as described by the manufacturer (Polytract™, Promega, Madison, WI). A cDNA library was constructed from 10 μg of ST40 poly(A)$^+$ RNA in the pSPORT1 vector using the Superscript™ plasmid system (Life Technologies Inc.). This library was screened by plaque hybridization with ^{32}P-labeled primer Muγ_1CH1 (5′-GAAATAGCCCTTGACCAGGCA-3′). This primer contains sequence information for the reverse complement of the murine γ_1 constant region gene, which codes for amino acids 142–148. The dideoxy chain termination sequencing of the V_H cDNA from selected clones was carried out on both strands using the T7 sequencing kit. The numbering of the amino acid sequences of variable regions was that of Kabat *et al.* (44).

Peptide Synthesis on Cellulose Membranes—The general protocol has been described previously (45). Membranes were obtained from Abimed (Langenfeld, Germany). Fmoc amino acids and *N*-hydroxybenzotriazole were obtained from Novabiochem (Läufelfingen, Switzerland). The ASP222 robot (Abimed) was used for the coupling steps. Two-hundred twenty overlapping dodecapeptides frameshifted by one residue representing the V_H and V_L sequences of the ST40 antibody were synthesized on cellulose membranes. All peptides were acetylated at their N termini. After the peptide sequences were assembled, the side chain-protecting groups were removed by trifluoroacetic acid treatment (41).

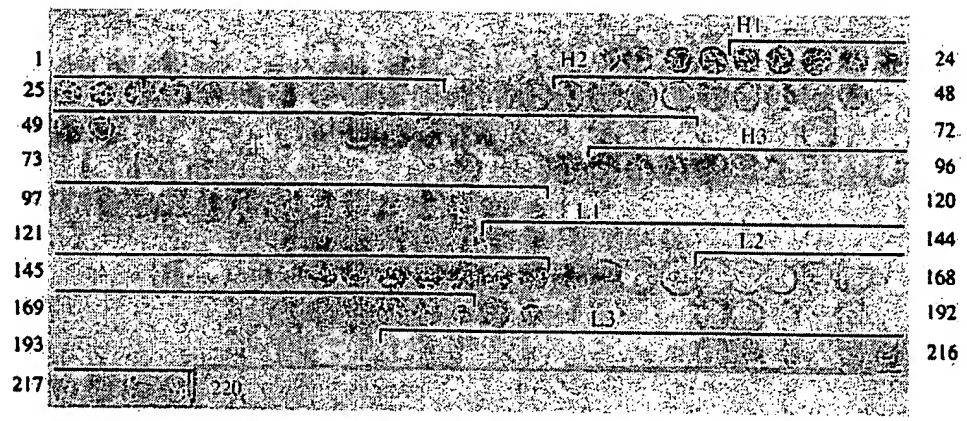
Assay for sCD4 Interaction with Cellulose-bound Peptides—The technique was performed as described previously for epitope analysis (41) and as adapted to paratope study (40). Briefly, the saturated membranes were incubated with a 1 μg/ml solution of biotinylated sCD4 for 90 min at 37 °C. Bound sCD4 was detected by incubation of the membrane at 25 °C for 30 min in a 1:3000 dilution of an alkaline phosphatase-streptavidin conjugate (Sigma) and subsequent addition of a phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate and 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, Sigma). A blue precipitate on the spots was indicative of binding. After scanning the membrane, NIH software was used to measure the spots' intensities (45). The membrane was further treated so as to remove precipitated dye and bound CD4 and reused when necessary. Inhibition of sCD4 binding to membrane-bound peptides was evaluated as described above, except that biotinylated sCD4 (1 μg/ml) was preincubated with anti-CD4 mAb ST40 (10 μg/ml) for 18 h at 4 °C.

Synthesis of Soluble Peptides and Cyclization—The 11 dodecapeptides, termed CM1–CM11 (see Table I), selected by the immunoassay described above, two control peptides (see below), and a CDR3-like peptide (TYICEVEDQKEE) corresponding to CDR3 loop 81–92 in D1 of CD4 were prepared by Fmoc solid-phase synthesis on a AMS422 robot. To improve solubility and to allow cyclization of peptides, Lys-Cys residues were added to both the carboxyl and amino termini of peptides CM1, CM2, CM6, CM7, and CM9–CM11. For peptides CM3–CM5 and CM8 and the CDR3-like peptide, the lysine residue was replaced by a tyrosine residue. The peptides were deprotected and released from the resin by trifluoroacetic acid treatment in the presence of appropriate scavengers. They were lyophilized, and their purity was assessed by HPLC. When necessary, the peptides were purified to >90% HPLC homogeneity. The peptides were cyclized by formation of a disulfide bond between the two extra cysteine residues as described by Tam *et al.* (46): 10 mg of peptide was dissolved in a solution of 20% dimethyl sulfoxide in 50 mM ammonium acetate buffer, pH 7.0, and stirred for 24 h at 20 °C. Peptide concentration was adjusted to 0.5 mg/ml to avoid the formation of intermolecular disulfide bonds. The efficiency of oxidation was assessed by determination of free sulfhydryl groups in the peptides (47). To this end, peptides (0.5 mg/ml, 10 μl) and 5,5′-dithiobis(2-nitrobenzoic acid) (0.4 mg/ml, 50 μl) were added to 100 mM Tris, pH 9.0, and the absorbance at 412 nm was determined and compared with the value obtained with the unoxidized peptides. Oxidation efficiency was further assessed by analytical HPLC by the change in the retention time of the oxidized peptide as compared with that of the linear form. The peptides showed >90% intramolecular disulfide bonding at the end of this procedure.

Enzyme-linked Immunosorbent Assay Monitoring of sCD4 and CDR3-like Peptide Interactions with Cyclic PDPs—Enzyme immunoassay plates (96-well; Nunc, Paisley) were coated overnight at 4 °C with 10-fold serial dilutions of the 11 cyclic PDPs (CM1–CM11) in 100 mM sodium carbonate buffer, pH 9.6. Three replicates were tested for each dilution with an initial peptide concentration of 100 μg/ml. An irrelevant cyclic peptide, 97026c (CKSSQSLLDSDGKTYLNWC), derived from the heavy chain CDR2 of an anti-p53 antibody was included as a control to verify that binding was sequence-specific. Two cyclic peptides, Dig23c (KCLEWIGDIYSGGGCK) and Dig97c (KCFGDYYCLQ-YASSCK), (derived from the heavy chain CDR2 and the light chain CDR3 of anti-digoxin mAb 1C10, respectively) were used as controls to verify the effect on antigen binding of adding Lys-Cys residues to the peptide sequence. After four washes in 160 mM PBS, pH 7.2, containing 0.1% Tween 20 (PBS-T), plates were saturated with a 1% nonfat powdered milk in PBS-T for 30 min at 37 °C. Biotinylated sCD4 (1 μg/ml) or biotinylated CDR3-like peptide (100 μg/ml) was added after four washes in PBS-T, and plates were incubated at 37 °C for 2 h. Following four washes in PBS-T, 100 μl of an alkaline phosphatase-streptavidin conjugate was added to each well. The conjugate was used at a 1:3000 dilution in PBS-T. The plates were incubated at 37 °C for 30 min and then washed four times in PBS-T. Finally, a 1 mg/ml 4-nitrophenyl phosphate disodium (Sigma) solution in 1 M diethanolamine, pH 9.8, was added for 20 min at 37 °C, and the absorbance was measured at 405 nm.

Real-time Analysis by BIAcore™—The kinetic parameters (association rate constant (k_a) and dissociation rate constant (k_d)) were determined by surface plasmon resonance analysis using a BIAcore instrument (BIAcore AB, Uppsala). Using BIAevaluation 3.0 software, k_a and k_d were determined by the so-called global method (48). The apparent equilibrium constant K_D is the ratio k_d/k_a. All experiments were carried out at 25 °C. Free NH_2 from the extrasequence lysine residue in CM1, CM2, CM6, CM7, and CM9–CM11 and from the intrasequence lysine residue in CM4 and free COOH from the glutamic acid residue in CM5 were used to chemically immobilize molecules on the sensor chip. Peptides CM3 and CM8 were chemically immobilized by the hydroxyl groups of threonine and serine, respectively, after activation by 1,1′-carbonyldiimidazole (Sigma-Aldrich). The surface plasmon resonance signal for immobilized peptides was found to be ~30–50 resonance units after completion of the chip regeneration cycle, corresponding to 30–50 pg of peptide/mm^2. The binding kinetics for immobilized peptides were determined by injecting sCD4 (20 μg/ml) in Hepes-buffered saline buffer (running buffer) at a flow rate of 30 μl/min. For the inhibition

A



B

	Peptide	Sequence	Color intensity of the spot (± SD)	
			Without preincubation	Preincubation with anti-CD4 mAb
ST40 V_H sequence	7	^{7}SGPELKKPGETV^{18}	6 ± 2	4
	15	^{15}GETVRISCKASG^{26}	66 ± 13	22
	16	^{16}ETVRISCKASGY^{27}	116 ± 21	31
	19 (CM1)	^{19}RISCKASGYGF^{30}	161 ± 14	12
	25 (CM2)	^{25}SGYGFTNAGMQW^{36}	162 ± 17	69
	29	^{29}FTNAGMQWVQKM^{40}	101 ± 17	35
	30	^{30}TNAGMQWVQKMP^{41}	35 ± 5	14
	37	^{37}VQKMPGKGLRWI^{48}	77 ± 12	22
	38	^{38}QKMPGKGLRWIG^{49}	98 ± 14	26
	47	^{47}WIGWINTHSGVP^{58}	92 ± 7	52
	48	^{48}IGWINTHSGVPT^{59}	11 ± 6	7
	49 (CM3)	GWINTHSGVPTY	131 ± 20	16
	50	WINTHSGVPTYA	106 ± 9	27
	51	^{51}INTHSGVPTYAE^{62}	17 ± 5	10
	55	^{55}SGVPTYAEDFKG^{66}	15 ± 2	4
	56	GVPTYAEDFKGR	80 ± 16	13
	59 (CM4)	TYAEDFKGRFAF	125 ± 10	17
	61	YAEDFKGRFAFS	100 ± 27	28
	61	^{61}AEDFKGRFAFSL^{74}	72 ± 18	29
	86	^{86}LKNEDTATYFCA^{97}	28 ± 5	18
	87	LKNEDTATYFCAR	124 ± 20	31
	89 (CM5)	NEDTATYFCARGG	139 ± 14	36
	99 (CM6)	GGVLWSRRGDFD	119 ± 15	57
	105 (CM7)	RRGDFDYWGQGT	145 ± 25	60
	106	RGDFDYWGQGTA	127 ± 23	43
	107	^{1003}GDFDYWGQGTAL^{1012}	79 ± 8	30
	108	DFDYWGQGTALT	121 ± 1	38
ST40 V_L sequence	121	^{1}DIELTQSPASLA^{12}	9 ± 4	4
	126	^{6}QSPASLAVSLGQ^{17}	28 ± 6	5
	127	SPASLAVSLGQR	93 ± 25	23
	128 (CM8)	PASLAVSLGQRA	122 ± 105	48
	133	VSLGQRATISCR	97 ± 5	25
	134	^{14}SLGQRATISCKA^{25}	77 ± 5	27
	148	^{27}QSVDYDGDSYMN^{38}	6 ± 1	4
	151	^{770}YDGDSYMNWYQQ^{781}	50 ± 9	19
	152	DGDSYMNWYQQK	145 ± 29	50
	154 (CM9)	DSYMNWYQQKPG	162 ± 23	96
	160	YQQKPGQPPKLL	100 ± 20	31
	161	^{33}QQKPGQPPKLLI^{46}	38 ± 10	12
	174	^{42}AASNLESGIPAR^{53}	19 ± 7	10
	175	ASNLESGIPARF	119 ± 23	35
	178 (CM10)	LESGIPARFSGS	165 ± 14	80
	182	IPARFSGSGSGT	150 ± 10	67
	183	^{52}PARFSGSGSGTD^{63}	47 ± 9	28
	211	^{61}YCQQSNEDPWTF^{72}	33 ± 8	13
	212	CQQSNEDPWTFG	55 ± 16	27
	213	^{62}QQSNEDPWTFGG^{73}	14 ± 4	14
	214	^{70}QSNEDPWTFGGG^{81}	17 ± 4	14
	215	^{71}SNEDPWTFGGGT^{82}	67 ± 16	19
	220 (CM11)	^{76}WTFGGGTKLEIK^{87}	143 ± 24	70

FIG. 1. **Reactivity of overlapping dodecapeptides derived from the sequence of anti-CD4 mAb ST40 with biotinylated sCD4 (*A*) and quantitative analysis of the binding (*B*).** The membrane on which the peptides were synthesized was incubated with 1 μg/ml biotinylated sCD4 or with 1 μg/ml biotinylated sCD4 preincubated with 10 μg/ml mAb ST40. In *A*, CDRs are indicated (*H1*, *H2*, and *H3* and *L1*, *L2*, and *L3* correspond to CDR1, CDR2, and CDR3 of the heavy and light chains, respectively), and peptide spots are numbered from 1 to 220. In *B*, *shaded* areas indicate the cellulose-bound peptides that reacted with biotinylated sCD4 (cutoff taken at 80 arbitrary units). *Boldface* amino acids belong to the CDRs. Results correspond to the mean ± S.D. of values obtained from three independent experiments.

study, mAb ST40 (20 μg/ml) and PDP (20 or 200 μg/ml) were co-injected onto the sensor chip-bound CD4 (30–50 pg/mm^2). The k_d increase was calculated as the ratio of k_d determined with inhibitor to that obtained without inhibitor.

HIV-1 Promoter Activation Assay—The HeLa P4 HIV-1 LTR β-galactosidase indicator cell line (49) was provided by O. Schwartz (Institut Pasteur, Paris). HeLa P4 cells, which stably express the β-galactosidase reporter gene cloned downstream of the HIV-1 LTR promoter, were plated in six-well plates at 5 × 10^5 cells/ml in Dulbecco's modified Eagle's medium containing a 1% penicillin/streptomycin mixture (Gibco), 1% Glutamax, 1 mg/ml Geneticin (G418), and 10% fetal calf serum. The cells were exposed to 1 ml of infectious HIV-1_{LAI} at 1000 × 50% tissue culture infective dose/ml prepared from the supernatant of chronically infected CEM T-cells, as described previously (50). After incubation for 1 h at 4 °C, the cyclic PDPs CM1, CM2, CM6, CM7, and CM9–CM11, at concentrations ranging between 12.5 and 200 μg/ml, were added individually to the cell culture medium. Next, cell cultures were transferred at 37 °C in a 5% CO_2 atmosphere to allow infection (note that the HIV-1 infection provides the viral transactivator Tat protein necessary for the HIV promoter in the target cells). After 3 days in culture, cells were lysed, and β-galactosidase activity was determined by incubating 200 μl of total cellular extracts for 1 h at 37 °C in 1.5 ml of buffer containing 80 mM Na_2HPO_4, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 6 mM *o*-nitrophenyl β-D-galactopyranoside. β-Galactosidase activity was evaluated by measuring absorbance at 410 nm. Incubation of infected HeLa P4 cells with anti-CD4 mAb ST40 at 20 μg/ml or anti-HLA class II mAb B8-12 (kindly provided by M. Hirn, Immunotech-Coulter, Marseille, France) at 20 μg/ml served as positive and negative controls, respectively. Additional controls consisted of linear Lyso-3 peptide (biotinyl-YKKSGTSPKRWIYDT), derived from the light chain CDR2 of anti-lysozyme mAb HyHEL-5 (40), and the cyclized 97026c peptide described above.

RESULTS

Sequence of Anti-CD4 mAb ST40—The nucleotide sequences of the V_H and V_L regions from anti-CD4 mAb ST40 were established as described under "Experimental Procedures." Nucleotide sequences of three individual clones were determined for each chain type and shown to be similar. Comparison of this sequence with other known antibody sequences showed that the V_H region of mAb ST40 belongs to subgroup IIA according to the classification of Kabat *et al.* (44) and displays 95.5% homology to the closest *VGK2* germ line gene (51) from the V-Gam 3.8 family. mAb ST40 used a member of the *DSP2* DH gene segment family, and the JH gene segment is homologous to the *JH2* germ line (44) except for a 3-nucleotide difference probably accounted for by somatic mutation. Sequence analysis suggests that the ST40 V_L region results from the rearrangement of a Vκ subgroup III gene with the *Jκ1* gene segment (44). More precisely, the ST40 V_L region shows 88% homology to the closest *Vκ21G* germ line gene (52) from the *Vκ21* family. Computer-assisted comparisons of these variable regions with other sequenced genes from anti-CD4 mAbs indicated that the ST40 V_L region shows strong homology to the V_L region of anti-CD4 mAb L71 (53). No significant homology to the anti-CD4 heavy chain has been found for the V_H sequence of mAb ST40.

Systematic Evaluation of the Reactivity of Overlapping Peptides from the ST40 Antibody Sequence with Biotinylated sCD4—Two-hundred twenty overlapping 12-mer peptides frameshifted by one residue, corresponding to the deduced amino acid sequences of V_H and V_L from mAb ST40, were synthesized according to the Spot method (40). These membrane-bound peptides were then probed with biotinylated sCD4. The results are described qualitatively in Fig. 1*A*, in which peptide spots showing at least one CDR residue are highlighted, and quantitatively in Fig. 1*B*. Biotinylated sCD4 bound mainly to peptides including amino acid(s) from the six CDRs of mAb ST40 (peptides 20–29, 31, 39–47, 49–50, 56–60, 88–106, 108, 133, 152–158, 175–180, 212, and 215–220). The majority of peptides containing only framework residues did not display any binding activities. However, several peptides (peptides 16–19, 38, 87, 127–132, 159–160, and 181–182) that contained only framework residues, mainly flanking the CDRs, were able to bind the sCD4 antigen. Little or no reactivity with sCD4 was observed with peptides comprising amino acids from the middle of the CDR sequence (peptides 30, 32–35, 48, 51–55, 61–66, 107, 134–151, 163–174, 202–211, and 213–214). As shown in Fig. 1*B*, this binding pattern was strongly affected by preincubating sCD4 with the parental anti-CD4 mAb ST40 (10 μg/ml). No binding was observed with the alkaline phosphatase-streptavidin complex alone (data not shown). Taken together and in accordance with previous observations (40), these results indicate that the binding of sCD4 to immobilized peptides is specific. Eleven peptides (peptides 19, 25, 49, 59, 89, 99, 105, 128, 154, 178, and 220, named CM1–CM11, respectively) showing the highest reactivity with sCD4 (color intensity of the corresponding spots between 119 ± 15 and 165 ± 14) were selected for further study in a soluble form. The selected peptides comprised either exclusively CDR (CM6) or framework (CM1 and CM8) sequences or comprised amino acids from both CDRs and framework sequences (CM2–CM5, CM7, and CM9–CM11).

TABLE I

BIAcore determination of the binding kinetics of the interaction between sensor chip-bound peptides derived from the V_H and V_L sequences of ST40 and biotinylated CD4

Boldface amino acids belong to the CDRs of mAb ST40.

PDP	Peptide sequence[a]	k_a	k_d	K_D
		$10^4\ s^{-1}\ M^{-1}$	$10^{-4}\ s^{-1}$	nM
CM1	KCRISCKASGYGFTCK	9.4	3.0	3.2
CM2	KCSGYGFTNAGMQWCK	13.7	2.2	1.6
CM3	YCGWINTHSGVPTYCY	7.7	16.9	22.0
CM4	YCTYAEDFKGRFAFCY	3.0	8.2	27.0
CM5	YCEDTATYFCARGGCY	10.3	3.5	3.4
CM6	KCGGVLWSRRGDFDCK	21.9	3.6	1.6
CM7	KCRRGDFDYWGQGTCK	22.4	7.2	3.2
CM8	YCPASLAVSLGQRACY	2.4	20.4	86.4
CM9	KCDSYMNWYQQKPGCK	8.5	9.1	10.6
CM10	KCLESGIPARFSGSCK	8.2	4.5	5.5
CM11	KCWTFGGGTYLEIKCK	8.1	3.7	4.6
97026c	CKSSQSLLDSDGKTYLNWC	NM[b]	NM	NM

[a] All peptides were cyclized through N- to C-terminal disulfide bridging.

[b] Not measurable.

CD4 and CDR3-like Loop Specificity of Soluble Cyclic Peptides Derived from the ST40 Antibody Sequence—The 11 peptides (CM1–CM11), selected from the initial 220 overlapping peptides on the basis of their reactivity with sCD4 in the form of membrane-bound peptides, were synthesized by conventional solid-phase synthesis and N to C terminus-cyclized through cysteine oxidation (Table I). Their binding to whole CD4 and to a CDR3-like loop peptide (corresponding to residues 81–92 in D1 of the CD4 molecule) was assessed by enzyme-linked immunosorbent assay (Fig. 2). Soluble cyclic peptides reacted specifically with sCD4 in a dose-dependent manner, which was not the case for the three irrelevant cyclic peptides 97026c, Dig23c, and Dig97c, the latter two including an extra lysine residue like the CM peptides. Peptides selected from either the V_H region (Fig. 2*A*) or the V_L region (Fig. 2*B*) displayed CD4 binding activity in a 1–100 μg/ml concentration range. Peptides CM2, CM6, and CM7 (Fig. 2*C*), derived from the ST40 V_H region, and peptides CM9 and CM11 (Fig. 2*D*), derived from the ST40 V_L region, strongly recognized CDR3-like peptide 81–92, whereas other synthetic peptides did not significantly bind this antigen. The linear forms of peptide CM9 and several other PDPs were markedly less reactive than the cyclic form (data not shown), indicating a beneficial effect of N- to C-terminal cyclization on binding properties. Furthermore, the absence of reactivity of the 12-mer Lys-Cys-cyclized

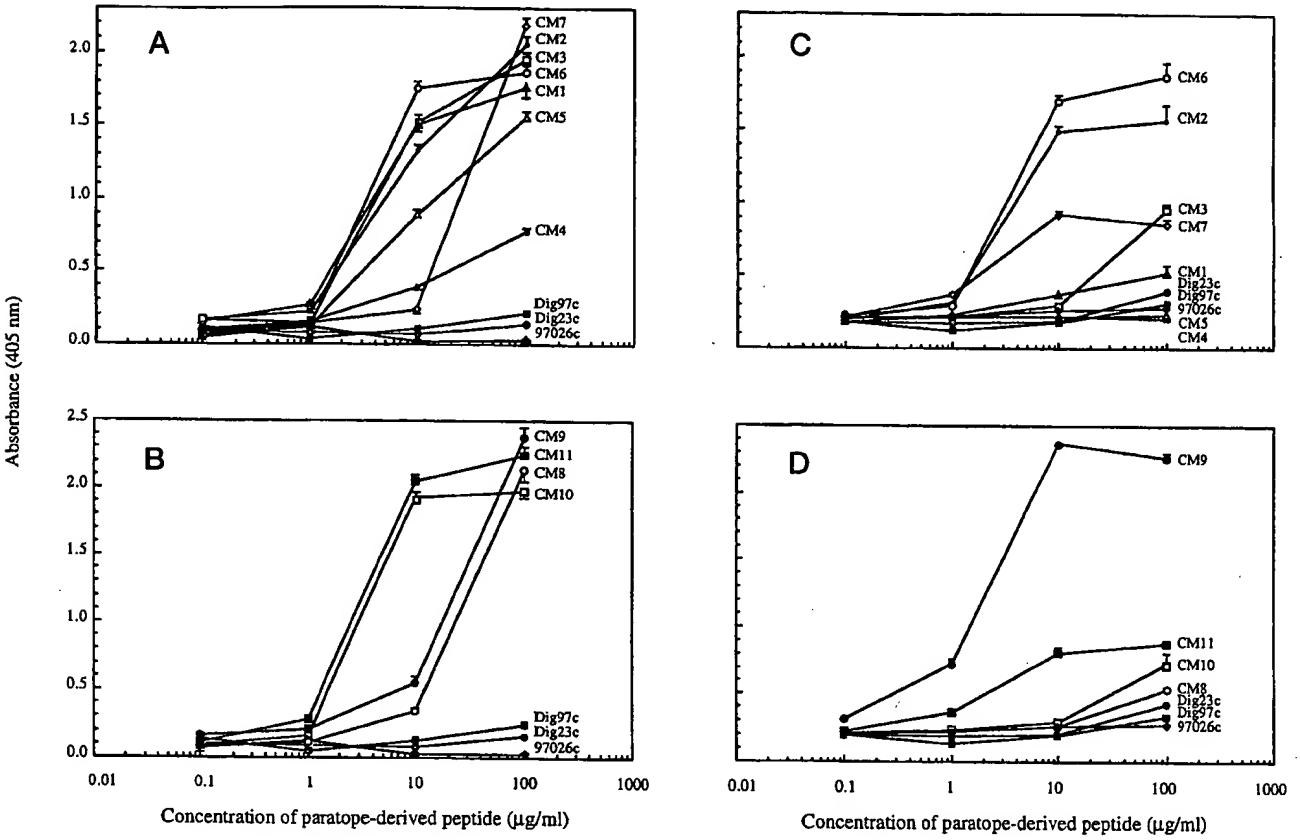


FIG. 2. **Enzyme-linked immunosorbent assay binding curves of biotinylated sCD4 or CDR3-like peptide 81–92 on adsorbed cyclic peptides derived from the sequence of mAb ST40.** Plates were coated with various concentrations of the cyclic peptides synthesized from the V_H sequence (*A* and *C*) and from the V_L sequence (*B* and *D*). Probing was performed either with biotinylated sCD4 (1 μg/ml) (*A* and *B*) or biotinylated CDR3-like peptide (100 μg/ml) (*C* and *D*). Irrelevant peptides (Dig23c, Dig97c, and 97026c) were used as negative controls. Each value represents the mean ± S.D. of triplicate determinations.

peptides Dig23c and Dig97c showed that the additional cysteine and lysine residues used for cyclization/solubilization are not implicated in the CD4 and CDR3-like binding. Taken together, these results indicate that the selected soluble cyclic peptides derived from mAb ST40 have the capacity to specifically bind the CD4 molecule, but only some of them also demonstrated a specificity for the CDR3-like loop.

The results of the BIAcore study, in which the kinetic parameters k_a and k_d of the interaction between immobilized peptides and soluble CD4 were measured, are summarized in Table I. All 11 peptides exhibited measurable binding to sCD4. No measurable binding was obtained with the irrelevant cyclic peptide. The calculated K_D values ranged from 1.6 to 86.4 nM. Peptides CM2 and CM5–CM7, derived from the CDR1 and CDR3 V_H regions of mAb ST40, showed the highest affinity. The K_D values obtained with the peptides showed a 4–8-fold increase in value as compared with the value obtained with the parental ST40 mAb (0.37 nM). This increase is mainly due to a lower dissociation rate of the mAb (0.33×10^{-4} s^{-1}) in comparison with that obtained with the PDPs.

Inhibition of HIV-1 Promoter Activation in Virus-infected Cells by PDPs—The ability of the PDPs to inhibit HIV-1 promoter activity was measured in HeLa P4 cells stably transfected with the β-galactosidase reporter gene under the control of the HIV-1_{Lai} LTR promoter. Infection of the indicator cell line with HIV-1_{Lai} strongly stimulated the HIV-1 promoter activity (mean $A_{410\,nm}$ increased from 0.014 to 0.548). As shown in Fig. 3*A*, no inhibition of the HIV-1 LTR-driven β-galactosidase gene expression was observed when HIV-1_{Lai}-infected indicator cells were cultured with anti-HLA class II mAb B8-12, whereas 65% inhibition was found following incubation with mAb ST40. Irrelevant linear and cyclic peptides did not affect the β-galactosidase gene expression. In contrast, treatment with the cyclic PDPs CM2, CM9, and CM11 significantly inhibited the HIV-1 LTR-driven β-galactosidase gene expression induced by HIV-1_{Lai}. Several other cyclic PDPs (CM1, CM6, CM7, and CM10) showed no effect. Peptide CM9, corresponding to the sequence ^{30}DSYMNWYQQKPG^{41} of the CDR1 framework-2 light chain region, was the strongest inhibitor. As shown in Fig. 3*B*, peptide CM9 inhibited, in a dose-dependent manner, the HIV-1 LTR-driven β-galactosidase gene expression induced by HIV-1_{Lai}. At a concentration of 63 μg/ml, peptide CM9 showed ~50% of the effect of the parental antibody used at 20 μg/ml. Taken together, these results indicate that the PDPs CM2, CM9, and CM11, initially selected among all the overlapping dodecapeptides of the V_H and V_L domains of anti-CD4 mAb ST40, are able to inhibit the HIV-1 promoter, a property previously ascribed to mAb ST40 (24).

Inhibition of ST40 Binding to CD4 by Three Paratope-derived Peptides—Competitive binding assays were performed to examine the ability of peptides CM2, CM9, and CM11 to block the binding of the parental ST40 mAb to sensor chip-bound CD4 (Table II). The three PDPs competed with the anti-CD4 antibody for binding to sensor chip-bound CD4, as determined by BIAcore analysis. This competition led to the enhancement of the dissociation rate of the antibody to the CD4 molecule. A 1000–2000-fold k_d increase was obtained when peptides were used at a concentration of 200 μg/ml. This inhibitory effect was

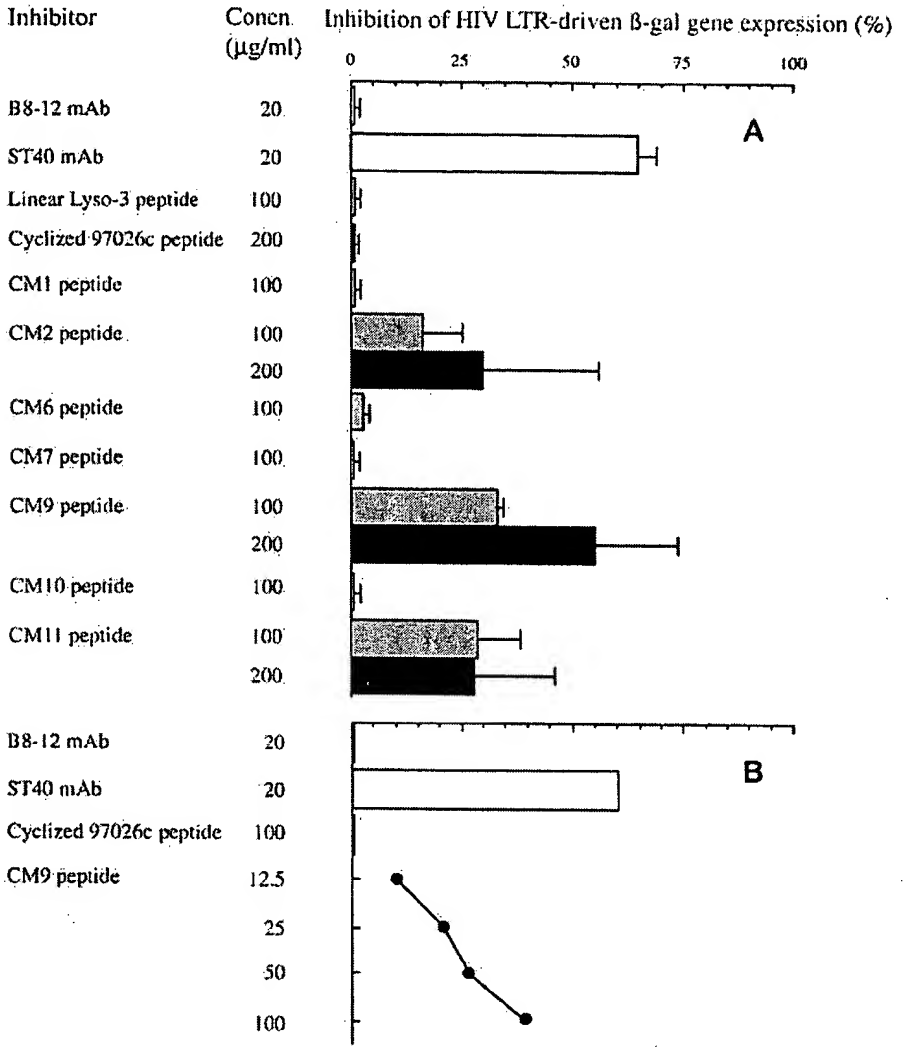


FIG. 3. **Inhibition of HIV LTR-driven β-galactosidase gene expression induced by HIV-1_{Lai} following incubation with cyclic PDPs synthesized from the ST40 sequence (*A*) and with different concentrations of peptide CM9 (*B*).** Irrelevant linear Lyso-3 and cyclic 97026c peptides were used as negative controls. Results correspond to the mean ± S.D. of inhibition values obtained from at least four independent experiments. Mean absorbances at 410 nm varied from 0.014 for uninfected indicator cells to 0.548 for HIV-1_{Lai}-infected indicator cells. *β-gal*, β-galactosidase.

TABLE II

Increase in the dissociation rate of the binding between sensor chip-bound CD4 and the anti-CD4 mAb co-injected with PDPs derived from the sequence of the antibody

Inhibitor	Conc	CD4/anti-CD4 interaction	
		k_d	-Fold increase
	μg/ml	$s^{-1}\ M^{-1}$	
None		3.3×10^{-5}	1
PDP			
CM2	20	1.2×10^{-3}	37
	200	2.6×10^{-2}	787
CM9	20	1.6×10^{-3}	48
	200	3.5×10^{-2}	1060
CM11	20	8.9×10^{-4}	27
	200	6.1×10^{-2}	1848

dose-dependent since a peptide concentration of 20 μg/ml caused only a 30–50-fold increase in the dissociation rate.

DISCUSSION

The identification, by using multiple peptide synthesis, of PDPs able to bind antigen was recently described by our group; several of these peptides display a significant fraction of the affinity of the whole antibody (40). Therefore, this approach could conceivably be used to screen peptide ligands mimicking the biological effect of a given antibody. With this perspective in mind, we have studied an anti-CD4 mAb (ST40) that shows interesting pharmacological activities. The ST40 antibody binds to the CDR3-like loop in D1 of CD4 and has been described as a strong inhibitor of HIV promoter activity and provirus transcription (24). We have established the V_H and V_L amino acid sequences of this antibody and assessed the reactivity of sCD4 with overlapping 12-mer peptides derived from these sequences by the Spot method (40, 41). Eleven peptides were found to react strongly and specifically with the CD4 antigen. We demonstrated that soluble cyclic peptides derived from peptides reactive in the Spot assay were able to recognize the CD4 molecule and a cyclic CDR3-like loop peptide corresponding to region 81–92 of CD4. Among the CDR3-like loop-specific PDPs, three (CM2, CM9, and CM11) were found to block HIV promoter activity and to compete efficiently with the parental mAb for binding to CD4.

An interesting feature was that PDPs showing the strongest reactivity with CD4 in the Spot assay included both residues from the CDRs and residues from the framework flanking the hypervariable regions, extending our previous observations (40). Antibody variable domains comprise a framework of β-sheets surmounted by antigen-binding loops. We can postulate that critical residues, identified in the Spot assay and confirmed by preliminary Alascan analysis (data not shown), located in the β-sheet framework closely underlying the CDRs, probably do not participate in direct interaction with CD4, but could induce a binding conformational state mimicking some of

the structural features of the ST40 paratope. Three points argue in favor of this hypothesis. First, some framework amino acids that modulate the peptide/CD4 interaction (*i.e.* Tyr^{27}, Trp^{47}, Gly^{49}, and Arg^{94} in the ST40 V_H sequence and Tyr^{36} in the ST40 V_L segment) belong to the vernier zone, which contains residues that adjust the CDR structure and fine-tune the fitting to the antigen (54). Second, some residues possess an aromatic structure (*i.e.* Tyr^{27} and Trp^{47} in the heavy chain and Tyr^{36} in the light chain) characterized as protruding into the antigen-binding site surface to stabilize the antigen/antibody interaction (55, 56). Third, framework arginine residues (*i.e.* Arg^{94} in V_H and Arg^{18} in V_L) modulate the peptide/CD4 binding, in keeping with previous work demonstrating the critical role of Arg^{94} in the interaction of a CDR3 V_H peptide with phosphatidylserine (36). These six critical residues from the framework regions of the ST40 antibody possess one or several of these characteristics, in agreement with previous results obtained in our laboratory on the interactions of mAb HyHEL-5/lysozyme (40) and mAb Tg10/thyroglobulin and mAb 4D8/angiotensin II.[2]

Based on the observations that CDR3-like synthetic peptides can bind CD4, Langedijk *et al.* (30) have proposed that the putative dimerization of CD4 involves the CDR3-like loop in D1. Moreover, electrostatic potential contours calculated for a putative CD4 dimerization occurring in D1 predicted that the negative electrostatic potentials of the CDR3-like region were completely compensated for by positive charges on the opposite CD4 molecule in the dimer (30). Recent results (29) suggest that Glu^{87}, Asp^{88}, Glu^{91}, and Glu^{92} in the CDR3-like loop are essential for CD4 dimerization and that these four negatively charged amino acids are involved in the ST40 epitope. These observations may have important implications for understanding how mAb ST40 interacts with CD4. We can speculate that positively charged residues from the CDRs of mAb ST40 could participate in the paratope. The cyclic peptides CM2, CM6, and CM7 from the V_H region and CM9 and CM11 from the V_L domain have been demonstrated to bind strongly to the CDR3-like loop of CD4 domain 1, and Lys-Cys residues added for cyclization/solubilization are not implicated in this binding. Positively charged residues, like Arg^{100G} and Arg^{100H} found in the sequence of the PDPs CM6 and CM7 from the CDR3 V_H region, Lys^{39} belonging to the sequence of peptide CM9 from the CDR1 V_L domain, and Lys^{107} in the PDP CM11, could conceivably interact with the negatively charged residues of the ST40 epitope. In agreement with this hypothesis, Arg^{100H} and Lys^{39} have been found to be critical amino acids by the Spot method in the peptide/CD4 interactions. Moreover, preliminary results obtained by Alascan analysis of PDPs confirm the contribution of these positively charged residues in CD4 binding (data not shown). However, positively charged amino acids probably reflect only a part of the interaction between ST40 and CD4 since other contributor residues in the CDRs were found by using Alascan analysis.

With regard to the measured binding kinetics of the interaction between soluble linear peptides from the HyHEL-5 paratope and lysozyme (40), a 1-log decrease in the k_d was observed in the peptide/CD4 binding, whereas association rates were in the same order of magnitude in the two models. In the case of anti-reovirus mAb 87.92.6 (34), it has been reported that the increased conformational stability of cyclic CDR peptides could increase the binding affinity. In addition, other reports (26, 57) suggest that cyclization helps peptides to mimic the CDR conformation. From these observations and from the results obtained with the CM peptide series, it seems that constraining the PDPs improves their affinity for antigen through a decrease in the dissociation rate of the equilibrium reaction between ligands. All the selected PDPs were able to bind sCD4 with K_D values ranging from ~2 to 90 nM, the best values being 4–8-fold higher than those obtained with the parental mAb.

mAb ST40 has been previously shown to inhibit HIV-1 LTR-driven chloramphenicol acetyltransferase gene expression induced by $HIV\text{-}1_{Lai}$ (24). The PDP CM9, derived from region 30–41 of the ST40 CDR2 V_L domain, blocks HIV promoter activity through the inhibition of β-galactosidase gene expression in a dose-dependent manner. The biological effect of CM9 was corroborated by further BIAcore experiments, in which this peptide was shown to displace the binding of ST40 to CD4 by increasing the rate of the dissociation reaction. Numerous bioactive peptides corresponding to the CDR3-like loop have been used to modulate the T-cell response (14, 17, 18) or to exert anti-HIV activity (26, 28). Disruption of CD4 dimerization by CDR3-like analogs has been proposed as a major mechanism by which cell activation could be inhibited following treatment of CD4-positive cells by CDR3-like analogs (18, 26, 29, 30). Furthermore, negatively charged residues in amino acid region 87–92 of CD4 can potentially be involved in the binding of a CDR3-like analog to CD4 (29). The facts that (i) the PDP CM9 interacts with CDR3-like region 81–92 and inhibits HIV-1 promoter activity and that (ii) residues 87/88 and 91/92 are involved in the epitope of the ST40 antibody, from which peptide CM9 has been designed, suggest that this PDP could act as an inhibitor of CD4 dimerization. Such an effect needs to be confirmed by additional experiments, even though we cannot rule out the fact that other CD4 regions might contribute to the oligomerization. Our results clearly demonstrate that the systematic exploration of sets of short cellulose-bound synthetic overlapping peptides derived from the sequences of immunoglobulin variable regions is a valuable strategy for identifying bioactive PDPs.

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A Monoclonal Antibody against the Platelet Fibrinogen Receptor Contains a Sequence That Mimics a Receptor Recognition Domain in Fibrinogen*

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The binding of fibrinogen to its platelet receptor, the glycoprotein IIb-IIIa complex, is mediated, in part, by an Arg-Gly-Asp (RGD) sequence within the fibrinogen Aα chain. PAC1 is an IgM-κ murine monoclonal antibody that binds to the platelet fibrinogen receptor, and its binding is inhibited by both fibrinogen and RGD-containing peptides. To identify the regions of PAC1 that interact with the fibrinogen receptor, we determined the mRNA sequences of PAC1 immunoglobulin heavy and light chain variable regions. Five out of the six complementarity-determining regions (CDRs) of PAC1 had entirely germline sequences with no regions of similarity to fibrinogen. However, CDR3 of the PAC1 heavy chain (H-CDR3) was very large and unique due to the insertion of a novel D region segment. H-CDR3 contained a sequence, Arg-Tyr-Asp (RYD), that, if present in the proper conformation, might behave like the RGD sequence in fibrinogen. A 21-residue synthetic peptide encompassing the H-CDR3 region inhibited fibrinogen-dependent platelet aggregation as well as the binding of PAC1 (K_i = 10 μM) and fibrinogen (K_i = 5 μM) to activated platelets. The RYD region of H-CDR3 appeared to be central to its function, because substitution of the tyrosine with glycine increased the inhibitory potency of the peptide by 10-fold, while replacing the tyrosine with D-alanine or inverting the RYD sequence sharply reduced the inhibitory potency. Thus, the linear sequence, RYD, within H-CDR3 of PAC1 appears to mimic the RGD receptor recognition sequence in fibrinogen. This type of immunologic approach could be useful in studying the structural basis of other receptor-ligand interactions.

Many adhesive proteins, including fibrinogen, fibronectin, von Willebrand factor, and vitronectin, contain a tripeptide, Arg-Gly-Asp (RGD),[1] that functions as a recognition sequence for adhesion receptors. The RGD sequences within these proteins are recognized by structurally related receptors on a variety of mammalian cells that have been termed integrins or cytoadhesins (1, 2). One well characterized member of the integrin family is the platelet membrane glycoprotein IIb-IIIa complex (GP IIb-IIIa) (3). Upon platelet activation, GP IIb-IIIa becomes competent to bind fibrinogen, a process required for platelet aggregation (4, 5). Based on studies with synthetic peptides, the RGDS sequence at position 572–575 of the fibrinogen Aα chain appears to play a major role in the interaction of this protein with GP IIb-IIIa, although other regions in fibrinogen may be involved as well (6–11). The precise details of the molecular interaction between fibrinogen and GP IIb-IIIa are unknown.

PAC1 is an IgM-κ murine monoclonal antibody that, like fibrinogen, binds to GP IIb-IIIa only on activated platelets (12). The binding of both PAC1 and fibrinogen is inhibited by RGD-containing peptides. For example, RGDS inhibits the binding of both ligands with an apparent K_i of 10–20 μM (6, 13). Moreover, PAC1 and fibrinogen compete with each other for binding to GP IIb-IIIa (13, 14). These data suggest that the antigenic site on GP IIb-IIIa for PAC1 is close to or identical with the binding site for fibrinogen.

The antibody combining region of PAC1 and other antibody molecules is contained within the variable regions of the heavy and light chains. Each heavy or light chain variable region is subdivided into four framework regions separated by three hypervariable or complementarity-determining regions (CDRs). X-ray diffraction studies of antibody molecules have shown that the antibody combining domain is comprised of all six CDRs, although each CDR may not be equally important in a given antibody molecule (15, 16). Recombination of gene segments allows for the formation of the variable region of an antibody and accounts, in part, for antibody diversity (17). For instance, any one of a few hundred variable (V) region segments may recombine with one of several diversity (D) region segments (within heavy chains) and one of several joining (J) region segments making a single variable region. In addition to combinatorial diversity, the CDRs accumulate somatically acquired point mutations that may enhance antibody affinity (18).

The similarity in the features of PAC1 and fibrinogen binding to GP IIb-IIIa could be based on three-dimensional similarity between the antibody combining region of PAC1 and the receptor binding domain(s) of fibrinogen. However, it is also possible that similarity exists at the level of primary structure. For example, Bruck and coworkers (19) have analyzed the sequence of an anti-idiotypic antibody reactive with

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04438 and J04439.

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[1] The abbreviations used are: RGD, Arg-Gly-Asp; CDR, complementarity-determining region; H-CDR3, CDR3 of the PAC1 heavy chain; RYD, Arg-Tyr-Asp; GP, glycoprotein; D, diversity; J, joining; V, variable; FITC, fluorescein isothyanate; dA, D-alanine; REDV, Arg-Glu-Asp-Val; RFDS, Arg-Phe-Asp-Ser; RYDS, Arg-Tyr-Asp-Ser; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

an antibody to reovirus and found sequence similarities between reovirus and two CDRs of the anti-idiotypic antibody. In the present study, as a first step in identifying the structural features of PAC1 and fibrinogen that account for their common specificity for GP IIb-IIIa, we have deduced the primary structures of the variable regions of the PAC1 heavy and light chains by determining the sequences of the mRNAs encoding these regions. We report here that the specificity of PAC1 for the platelet fibrinogen receptor is determined largely by the amino acid sequence within CDR3 of the PAC1 heavy chain. In particular, a tripeptide sequence within this CDR, Arg-Tyr-Asp (RYD), is responsible for this activity, analogous to the activity of RGD within the fibrinogen molecule.

MATERIALS AND METHODS

Cell Culture and Isolation of mRNA—PAC1 hybridoma cells were grown in RPMI 1640/10% fetal calf serum, and 5×10^8 cells were harvested and resuspended in 4 M guanidine thiocyanate (20). Total RNA was isolated after centrifugation through a CsCl cushion and poly(A^+) RNA was isolated using oligo(dT)-cellulose (21).

Sequencing of PAC1 Immunoglobulin Variable Regions—Using complementary oligonucleotide primers from the 5′ region of the mouse μ and κ constant region mRNAs, (5′-GCTCTCGCAGGAGACGAG and 5′-GGTGGGAAGATGGATACAGTT, respectively), the sequences of PAC1-μ and -κ mRNAs were determined using the direct dideoxy chain termination method as described by Geliebter (22). Briefly, 20 ng of end-labeled primers were annealed to 10 μg of poly(A^+) mRNA at 50 °C and primers were extended in the presence of dideoxynucleotides and reverse transcriptase (Amersham Corp.). The products of this reaction were electrophoresed on 6% denaturing polyacrylamide gels that were then exposed to autoradiography. Once approximately 200 base pairs of sequence had been determined, complementary primers within the variable region about 150 base pairs upstream of the constant region were synthesized in order to extend the sequence further 5′ within the variable region. The primer used in the PAC1-μ reaction was 5′-CATTAGCTTGCAGACTGTTC and in the PAC1-κ reaction, 5′-ATCTTGAGTGTGAAATCTGT. A small portion, about 50 nucleotides, of the 3′ end of the κ mRNA was determined using the modified Maxam and Gilbert technique of Schlomchik *et al.* (23). Sequences were analyzed and regions of amino acid similarity between fibrinogen and PAC1 identified using Microgenie (Beckman) and Intelligenetics software. Protein secondary structure was predicted on Microgenie using the algorithm of Garnier *et al.* (24).

Peptide Synthesis and Analysis—The peptide H-CDR3-RYD (Ala-Arg-Arg-Ser-Pro-Ser-Tyr-Tyr-Arg-Tyr-Asp-Gly-Ala-Gly-Pro-Tyr-Tyr-Ala-Met-Asp-Tyr-amide) and analogs were synthesized by the solid-phase method using an Applied Biosystems 430A automated synthesizer. *t*-Butoxycarbonyl-amino acids supplied by the manufacturer were used except for *t*-butoxycarbonyl-Asp (cyclohexyl). Triple couplings were used for Arg^{100A}, Ser^{98}, Tyr^{99}, Tyr^{100}, Arg^{95}, Arg^{94}, and Ala^{93}; the remaining residues were incorporated by a double coupling protocol. The peptides were cleaved from the solid support by the Sn2/Sn1 HF cleavage procedure (25) and purified by gel filtration (Sephadex G-50F, 50% HOAc) and reverse-phase high performance liquid chromatography (Vydac C_{18}, 15 μm, 5 × 30-cm column, 95% A-B to 75% A-B over 60 min at a flow rate of 100 ml/min; A = 0.1% trifluoroacetic acid-H_2O, B = 0.1% trifluoroacetic acid-CH_3CN). Products were characterized by amino acid analyses after acid hydrolysis, fast atom bombardment mass spectrometry, nuclear magnetic resonance spectroscopy, and sequence analysis after Edman degradation. Analyses were consistent with the expected structures and with product purity of ≥95%.

Binding of PAC1 and Fibrinogen to Platelets—Monoclonal antibody AP1, specific for platelet membrane glycoprotein Ib, was a gift from Dr. Thomas Kunicki, Blood Center of Southeastern Wisconsin (26). Antibody S12, specific for an α granule membrane protein (GMP-140) expressed on the surface of activated platelets, was a gift from Dr. Rodger McEver, Oklahoma Medical Research Foundation (27). Antibody 9F9, specific for fibrinogen, was a gift from Dr. Andrei Budzynski, Temple University (28). Antibody B1B5 is an antibody generated in our laboratory specific for glycoprotein IIb. Antibodies were purified as described previously (12, 29).

The binding of PAC1 and other anti-platelet monoclonal antibodies as well as fibrinogen to gel-filtered human platelets was examined by fluorescence-activated flow cytometry (30). Venous blood from normal donors was drawn into 1/7 volume of NIH formula A acid-citrate-dextrose solution, platelet-rich plasma was obtained, and the platelets were gel-filtered into an isotonic HEPES-containing buffer at pH 7.4 (14). To examine the effect of synthetic peptides on PAC1 binding to activated platelets, 5×10^7 platelets were incubated for 15 min at room temperature in a final volume of 50 μl in the presence of 0–100 μM peptide, 40 μg/ml fluorescein-labeled PAC1 (FITC-PAC1), and 10 μM ADP and epinephrine. Then 500 μl of the isotonic buffer was added and antibody binding was quantitated using a FacStar flow cytometer (Becton Dickinson), as described previously (30). Fibrinogen binding to platelets was measured using the same incubation system except that 50 μg/ml of purified human fibrinogen (4) was added and the monoclonal antibody FITC-9F9 was used instead of FITC-PAC1. 9F9 is specific for fibrinogen and has been used successfully to measure the amount of fibrinogen bound to platelets (28). Previous studies have established that the amount of FITC-labeled antibody bound to platelets determined by flow cytometry is related in a linear fashion to the amount of antibody bound determined in a conventional radioligand binding assay (30). The amount of platelet-bound FITC-PAC1 or FITC-9F9 was determined by analyzing 10,000 platelets for the extent of fluorescence at 488 nm, and binding is expressed as the mean fluorescence intensity in arbitrary fluorescence units.

Platelet Aggregation Studies—The aggregation of gel-filtered platelets was carried out as described previously (14). Platelets (2×10^8/ml) were stirred at 37 °C in the presence of human fibrinogen (100 μg/ml) and $CaCl_2$ (1 mM) and aggregation was initiated by the addition of 10 μM ADP. Aggregation was monitored as a change in light transmittance, and is expressed as the initial rate of aggregation.

Rheumatoid Factor Assay—PAC1 was screened for rheumatoid factor activity in a solid-phase radioimmunoassay, essentially as has been described (31). A murine IgM with known rheumatoid factor activity (VS1) was used as a positive control and was a gift from Dr. Martin Weigert, Fox Chase Cancer Center. Another murine IgM (87.92.6.11) served as a negative control and was a gift from Dr. Mark Greene, University of Pennsylvania. Murine IgG1 served as the solid-phase antigen and was bound to Immulon II microtitre wells by adding 200 μl of 20 μg/ml affinity-purified IgG1 (Sigma) and incubating overnight at 4 °C, pH 9.0. After washing and then blocking for 2 h with 1% bovine serum albumin in phosphate-buffered saline, pH 7.4, 150-μl aliquots of sequential dilutions of PAC1, VS1, or 87.92.6.11 were added for 2 h at room temperature. After washing, 200 μl of affinity-purified, ^{125}I-labeled goat anti-mouse μ chain-specific antibody (Zymed; 100,000 cpm/well; 900 cpm/ng) were added for 2 h at room temperature. After washing, the wells were dried and counted for ^{125}I in a γ counter.

RESULTS

The monoclonal antibody PAC1 appears to be specific for the fibrinogen binding site on platelet GP IIb-IIIa (12, 13). As a first step in characterizing the structural features of PAC1 responsible for its interactions with GP IIb-IIIa, we sequenced both the heavy and light chain variable regions of PAC1 immunoglobulin mRNA. We reasoned that linear sequence(s) of amino acids within the CDRs of the PAC1 antibody might be important for its binding to platelets, and that an analysis of the antibody variable region sequences might allow us to identify those binding regions.

The CDRs of the PAC1 Light Chain Contain No Sequences With Similarity to Fibrinogen—Fig. 1 shows the nucleotide sequence of the variable region of the κ light chain along with the predicted amino acid sequence. The CDRs are *boxed* and the amino acids numbered according to convention (15). This κ variable region is a class II mouse κ variable region. Although its sequence is not identical to any germline variable regions present in the data bank, only a small number of germline variable regions have been sequenced. The only similarity between PAC1-κ and fibrinogen was found in framework 3 of PAC1-κ and the γ chain of fibrinogen. Two short stretches of PAC1-κ, amino acids 60–62 and 85–88, were identical to amino acids 320–322 and 346–349, respectively, on the fibrinogen γ

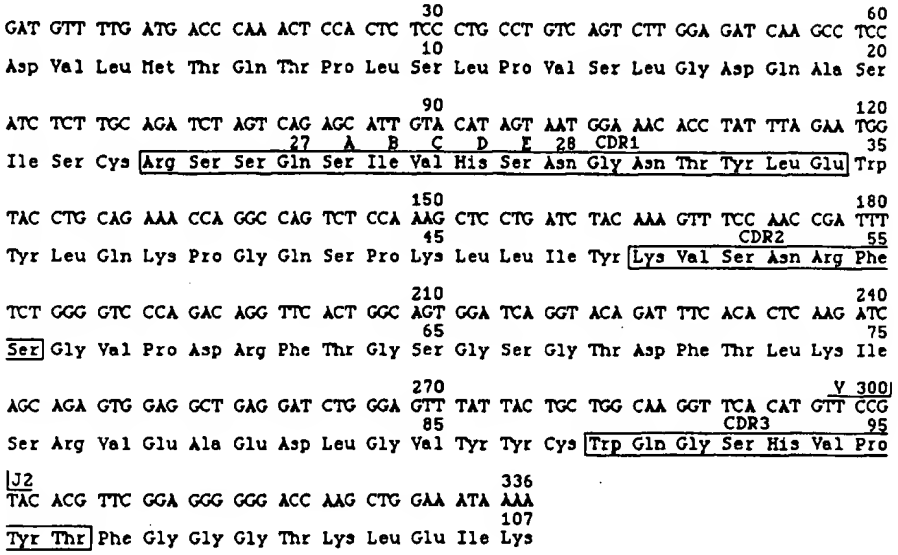

FIG. 1. **Sequence of PAC1-κ light chain variable region.** The nucleotide sequence of the PAC1-κ variable region is shown with the numbering above. The site of the V to J (J2-κ) region junction is indicated. The amino acid numbers and translation of the nucleotides appear on the next two lines. The amino acids are numbered according to convention (15), and the regions corresponding to CDRs are indicated and *boxed*.

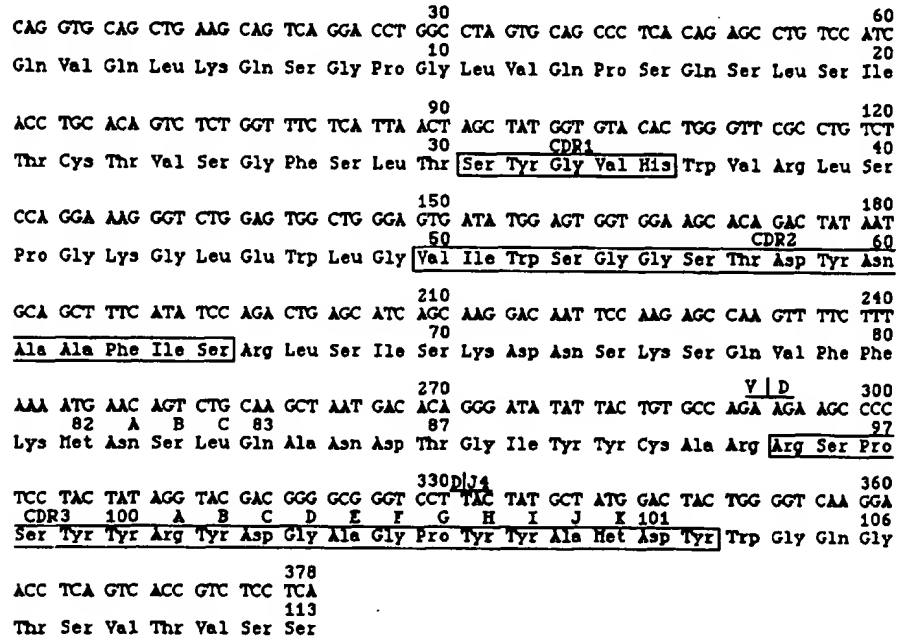

FIG. 2. **Sequence of PAC1-μ heavy chain variable region.** The sites of V to D and D to J (J4) region junctions are indicated.

chain with the exception of two basic amino acids: an arginine at position 61 in PAC1-κ and a lysine at position 321 in fibrinogen. There were no apparent similarities between the intervening amino acids in PAC1 (63–84) and fibrinogen (323–345).

Further analysis showed that with the exception of one conservative amino acid difference in the J region segment and two other conservative amino acid differences in the framework region of the V region, PAC1-κ was identical to the κ variable regions of two mouse rheumatoid factors, VS1 and JV6 (23). These rheumatoid factors are felt to be naturally occurring nonpathologic, germline IgM immunoglobulins that bind to IgG (23). It is not known whether the rheumatoid factor binds to IgG through its hypervariable or framework regions. Because of the near identity of PAC1-κ with VS1-κ and JV6-κ and the above noted similarity between fibrinogen and a PAC1-κ framework region, two experiments were performed to test the relationship between PAC1 and rheumatoid factors. First, PAC1 was tested for rheumatoid factor activity against murine IgG_1 in a solid-phase radioimmunoassay and was found to be negative. Second, the known rheumatoid factor, VS1, was conjugated with fluorescein and tested by flow cytometry for its ability to bind to resting or ADP-stimulated, gel-filtered platelets. Despite the fact that the light chain of VS1 is virtually identical to that of PAC1, no specific binding of VS1 to platelets was observed (data not shown).

CDR3 of the PAC1 Heavy Chain Contains a Region of Potential Similarity with Fibrinogen—The sequence of the PAC1 heavy chain region is shown in Fig. 2. The portion of this variable region derived from the V region segment and containing CDRs 1 and 2 is identical, with the exception of two amino acids in the framework 3 region, to the germline VH101 variable region, a subgroup I (*B*), mouse heavy chain variable region. Within this V region segment, no sequence similarity to fibrinogen was detected. However, CDR3 of PAC1-μ is extremely long due to the insertion of a novel D region segment. A portion of this region inclusive of nucleo-

tides 302–318 is identical to the D region segment Dsp2–6 except for the nucleotide triple encoding the arginine (amino acid 100A). Apparently somatic mutations have occurred at this position which encodes a glycine in the germline Dsp2–6 segment. On either side of this Dsp2–6 segment in PAC1, so-called N region segments are found. These sequences are a result of either aberrant recombination or the addition of novel nucleotides next to the D region segment (32) and have no significant homology to D region segments on file in GenBank.

Although detailed computer-based modeling of the PAC1 H-CDR3 region was not performed due to its lack of similarity to H-CDR3 regions that have been studied crystallographically, a simple algorithm was used to predict basic secondary structure (24). Like most CDRs, the basic structure is compatible with an antiparallel β loop (15). The β strand region ends abruptly at amino acids 97 and 100G, 2 proline residues that may result in a looping out of amino acids 98 through 100F. Of note, the center of this putative loop (100A–C) contains the sequence Arg-Tyr-Asp (RYD). The arginine within the tripeptide was derived through somatic mutations as noted above. This RYD sequence in PAC1 could potentially interact with the RGD binding site on platelet GP IIb-IIIa if the side chain of the tyrosine residue were in the proper conformation, presumably directed away from the side chains of arginine and aspartic acid. In addition, Ser^{96}, Ser^{98}, and Tyr^{99} of H-CDR3 are identical to residues at the same positions preceding the $RGD^{572-575}$ of fibrinogen Aα.

A Synthetic Peptide Derived from PAC1 H-CDR3 Inhibits the Interaction of PAC1 and Fibrinogen with Human Platelets—To test the prediction that the RYD sequence within H-CDR3 of PAC1 plays a role in the binding of PAC1 to the platelet fibrinogen receptor, we synthesized as 21-residue peptide (referred to as H-CDR3-RYD) that includes amino acids 93–102 of the PAC1-μ variable region (Fig. 2), and tested its ability to inhibit the binding of PAC1 and fibrinogen to activated platelets. Gel-filtered platelets were stimulated maximally with ADP and epinephrine (10 μM each) for 15 min in the presence of a subsaturating concentration of FITC-PAC1 and various concentrations of H-CDR3-RYD. Then, the amount of FITC-PAC1 bound to the platelets was determined by flow cytometry. Under these conditions, the H-CDR3-RYD peptide inhibited the binding of FITC-PAC1 to platelets with an IC_{50} of 20–30 μM (Fig. 3*A*). This was similar to the IC_{50} of the tetrapeptide, RGDS. In five separate experiments, Dixon plot analysis (33) revealed that the apparent K_i for inhibition of PAC1 binding by H-CDR3-RYD was 10.2 ± 4.3 μM (S.E.). As a control, an unrelated peptide of similar length had no effect on FITC-PAC1 binding (Fig. 3*A*). Identical results were obtained when platelets were activated with phorbol myristate acetate instead of ADP and epinephrine.

Since PAC1 binds to an epitope on GP IIb-IIIa at or near the binding site for fibrinogen, we also tested the ability of H-CDR3-RYD to inhibit fibrinogen binding to activated platelets. Fibrinogen binding was assessed by flow cytometry using a FITC-labeled anti-fibrinogen monoclonal antibody, 9F9. When platelets were activated with ADP plus epinephrine in the presence of 50 μg/ml fibrinogen, H-CDR3-RYD inhibited fibrinogen binding with an IC_{50} of 10–20 μM (Fig. 3*B*). In three experiments, Dixon plot analysis showed that

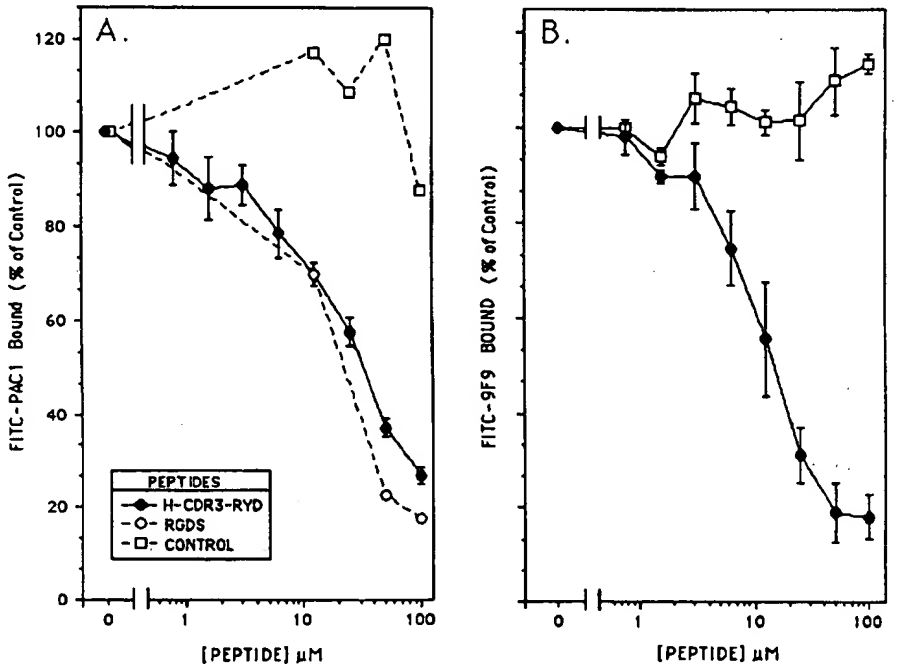

FIG. 3. **Effect of the H-CDR3-RYD peptide on the binding of PAC1 and fibrinogen to activated platelets.** As described under "Materials and Methods," gel-filtered platelets were incubated for 15 min with ADP (10 μM), epinephrine (10 μM), the indicated peptides, and either FITC-PAC1 (*A*) or FITC-9F9 and fibrinogen (*B*) (50 μg/ml). Then PAC1 and fibrinogen (*9F9*) binding were determined by flow cytometry. The peptides studied in this experiment included: H-CDR3-RYD, the synthetic 21-residue peptide encompassing amino acids 93–102 of the PAC1-μ variable region (see Fig. 2); RGDS, Arg-Gly-Asp-Ser; and a "control peptide" similar in length to H-CDR3-RYD but with an unrelated sequence, YVDGDQC(Acm)ESNPCLNGGM(O)C(Acm)KDDINSYGCF-amide. Antibody binding is expressed as the percentage of binding observed in the absence of an added peptide. *Error bars* represent the mean ± S.E. of five separate experiments. In *A*, the data points without error bars represent the mean of two experiments.

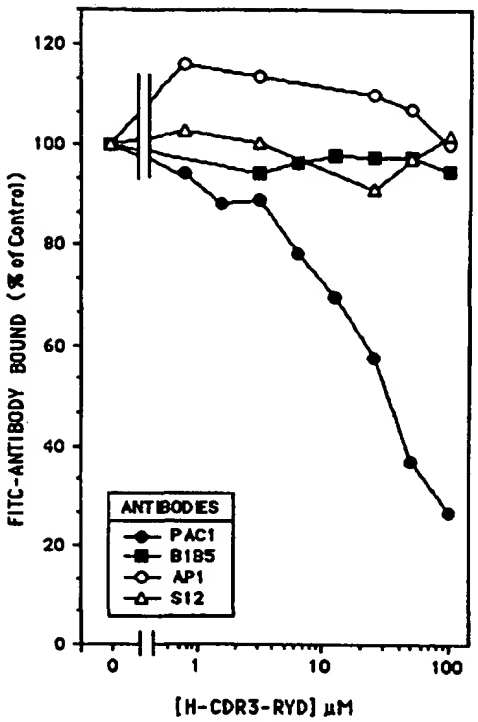

FIG. 4. **Effect of the H-CDR3-RYD peptide on the binding of various monoclonal antibodies to platelets.** As in Fig. 3, gel-filtered platelets were incubated in the presence of H-CDR3-RYD and various FITC-labeled, anti-platelet monoclonal antibodies, and then monoclonal antibody binding was determined by flow cytometry. The specificity of each of the antibodies is indicated in the text. In the case of antibody AP1, the platelets were not activated. In the case of antibodies PAC1, B1B5, and S12, the platelets were activated with ADP and epinephrine (10 μM each). The data are from a single experiment representative of two so performed.

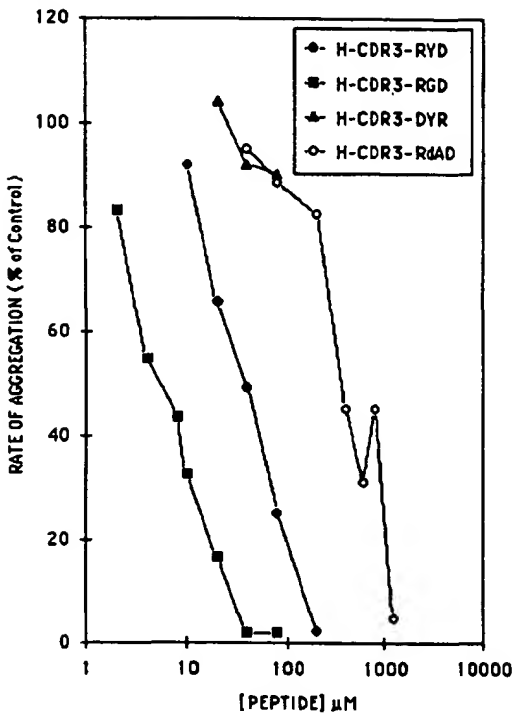

FIG. 5. **Effect of H-CDR3-RYD and related peptides on platelet aggregation.** Gel-filtered platelets were stirred for 3 min in an aggregometer cuvette in the presence of ADP (10 μM), fibrinogen (100 μg/ml), $CaCl_2$, (1 mM), and the indicated peptides. Aggregation is expressed as the initial rate of aggregation where the rate of aggregation in the absence of added peptide is arbitrarily denoted as 100%. The data represent the means of three separate experiments.

this peptide inhibited fibrinogen binding with an apparent K_i of 5.5 ± 3.5 μM.

To examine the specificity of the H-CDR3-RYD peptide as an inhibitor of PAC1 binding, the effect of this peptide on the binding of monoclonal antibodies to other platelet glycoproteins was examined. The H-CDR3-RYD peptide had no effect on the binding of FITC-AP1 to platelet glycoprotein Ib on resting platelets. In addition, the peptide had no effect on the binding of either FITC-B1B5 to glycoprotein IIb or FITC-S12 to GMP-140 on activated platelets (Fig. 4).

Since agonist-induced platelet aggregation is dependent on fibrinogen binding, and since PAC1 inhibits platelet aggregation, we examined the effect of H-CDR3-RYD on aggregation. In three experiments in which gel-filtered platelets were stirred in the presence of 100 μg/ml fibrinogen, H-CDR3-RYD inhibited the initial rate of ADP-induced platelet aggregation with an IC_{50} of approximately 40 μM (Fig. 5). At 10 μg/ml fibrinogen the IC_{50} was approximately 10 μM (not shown).

Modifications in the RYD Sequence of the H-CDR3 Peptide Affect Its Inhibitory Activity—The specific role of the RYD sequence within H-CDR3-RYD was examined by testing three additional peptides identical to H-CDR3-RYD except for specific modifications in the RYD region. Substitution of the tyrosine in RYD with glycine (H-CDR3-RGD) increased the inhibitory potency of the H-CDR3 peptide by about 10-fold in both the aggregation assay (Fig. 5) and the PAC1 and fibrinogen binding assays (Fig. 6). On the other hand, substitution of the tyrosine with a D-alanine (H-CDR3-RdAD) or inversion of the R and D residues (H-CDR3-DYR) substantially reduced the inhibitory capacity of the peptide.

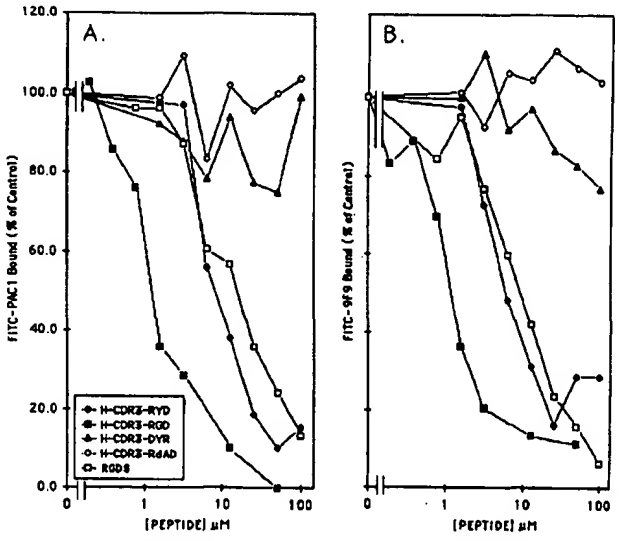

FIG. 6. **Effect of H-CDR3-RYD and related peptides on the binding of FITC-PAC1 (*A*) or FITC-9F9 (*B*) (*i.e.* fibrinogen) to platelets stimulated with ADP and epinephrine (10 μM each).** The conditions in each of the binding assays were identical to those in Fig. 3. Data are from a single experiment representative of three so performed.

These results indicate that the primary structure of the CDR3 of the PAC1 heavy chain plays a major role in the binding of this antibody to the fibrinogen receptor on activated platelets. In particular, the RYD sequence within this CDR appears to mimic the RGD sequence in fibrinogen,

thereby conferring specificity of this antibody for the platelet fibrinogen receptor.

DISCUSSION

Several lines of evidence indicate that the epitope for the PAC1 monoclonal antibody on the platelet GP IIb-IIIa complex is at or very near the binding site for fibrinogen: 1) PAC1 binding to GP IIb-IIIa, like fibrinogen binding, is dependent on platelet activation (4, 5, 12); 2) PAC1 and fibrinogen compete with each other for binding to platelets (13, 14); and 3) the binding of both PAC1 and fibrinogen is inhibited by small peptides containing the core sequence RGD (6, 7, 13). As further proof of the specificity of this interaction, anti-idiotypic antibodies raised to PAC1 exhibit binding properties similar to GP IIb-IIIa in that they interact with both PAC1 and fibrinogen.[2] In the present study, we have determined the amino acid sequences of the heavy and light chain variable regions of PAC1 to see if we could identify linear sequences within the CDRs of the antibody and similar sequences within fibrinogen that are critical for binding to GP IIb-IIIa.

The structural features of the PAC1 variable region sequences allowed us to successfully identify a sequence within H-CDR3 that is critical for PAC1 binding to GP IIb-IIIa. Like many IgM antibodies, PAC1 has not accumulated a large number of somatic mutations in its CDRs (34, 35). Five out of the six CDRs of PAC1 are found in germline variable regions. These CDRs are not eliminated as necessary regions in the antibody combining site, because several IgM antibodies with few or no somatic mutations within the CDRs have been shown to exhibit antigen specificity (34, 35). However, since somatic mutations may greatly enhance antibody affinity (18), identifying a CDR that contains somatic mutations may help pinpoint an important region in the antibody combining domain. Because these five PAC1 CDRs lack either somatic mutations or sequence similarity with fibrinogen, we focused our attention on H-CDR3, the one PAC1 CDR that is not in a germline configuration. Given our findings with H-CDR3 (discussed below), it may be that portions of the other PAC1 CDRs function in stabilizing the interaction between H-CDR3 and GP IIb-IIIa.

Two characteristics of the PAC1 H-CDR3 sequence suggested to us that this CDR has a major role in determining the binding specificity of PAC1. First, due to the occurrence of somatic point mutations in the D region segment, PAC1 H-CDR3 contains a sequence, RYD, that has potential similarity to the RGD sequence in fibrinogen. Secondary structure analysis suggested that a loop-like structure conferred by 2 surrounding proline residues might make the RYD available for binding to GP IIb-IIIa. Second, because of the presence of N region segments, H-CDR3 is very long, supporting its involvement in the antibody combining domain of PAC1. In x-ray diffraction studies of the antibody KOL, the long H-CDR3 region was shown to occupy a large portion of the antibody binding domain (36, 37). Since antibodies have very similar three-dimensional structures, by analogy with KOL, the long H-CDR3 region within PAC1 should also occupy a large portion of the PAC1 binding domain (38).

To test the role of H-CDR3 in PAC1 binding to the platelet fibrinogen receptor, we synthesized a 21-residue peptide encompassing the entire H-CDR3 region. This peptide specifically inhibited the binding of both PAC1 and fibrinogen to activated platelets with a K_i of about 10 μM, a value similar to the K_i for the tetrapeptide RGDS (6, 13). The H-CDR3 peptide also inhibited platelet aggregation, a process that requires fibrinogen binding to platelets. Specific modifications of the RYD sequence within the H-CDR3 peptide clearly established that this sequence of three amino acids is critical for the functional properties of the entire peptide. On the basis of this information, we conclude that the ability of PAC1 to bind to the platelet fibrinogen receptor is dependent, in large part, on the similarity of the RYD sequence within H-CDR3 to the RGD sequence within the Aα chain of fibrinogen. Of the H-CDR3-derived peptides that had been modified in the RYD region, the peptide in which a glycine had been substituted for the tyrosine (H-CDR3-RGD) was 10-fold more effective than H-CDR3-RYD in inhibiting the interaction of PAC1 and fibrinogen with platelets. Presumably, the glycine residue in the substituted peptide is in a more favorable conformation for binding to GP IIb-IIIa than the tyrosine residue within H-CDR3-RYD. The conformation of the RYD region in the intact antibody molecule is likely to be optimized for binding to GP IIb-IIIa through contact with other regions in PAC1. Based on the results with these peptides, it is also possible that a glycine-for-tyrosine substitution in the intact antibody might increase the antibody's already high apparent affinity (5 nM) even further.

In many of the adhesive proteins reported to bind to the integrin family of adhesion receptors, it appears that each amino acid of the RGD core is critical for optimal binding activity (2, 39). In PAC1, the three-dimensional structure of the RYD-containing, antigen-binding domain may be sufficiently similar to the RGD-containing domain of fibrinogen to permit tight interaction of the antibody with GP IIb-IIIa. Other ligands with an RGD-like sequence may bind to adhesion receptors in some systems. For example, a region of alternatively spliced fibronectin containing the sequence REDV has been implicated in the binding of fibronectin to melanoma cells (40). It is of interest that both RFDS and RYDS are highly conserved in major histocompatibility antigens and are putative cellular adhesion sites (41). Furthermore, von Willebrand factor, which can bind to GP IIb-IIIa on activated platelets, contains an RYD as well as an RGDS sequence (42). In these latter cases, the role, if any, of the RYD sequence in cell-cell or protein-cell interactions remains to be determined.

Using computer modeling based on known structures of crystallized antibodies, the three-dimensional structures of the variable regions of antibodies are now routinely predicted. However, computer-based modeling may result in errors or ambiguities leading to incorrect structural predictions (43). Because the CDRs of PAC1 have low similarity to the CDRs of antibodies studied crystallographically and are, therefore, unfavorable for modeling, we chose to examine the primary structure of the variable regions of PAC1, particularly the CDRs, for linear sequences with similarity to fibrinogen. Using this approach we were successful in identifying a region within one of the CDRs that confers specificity of PAC1 for its antigen, GP IIb-IIIa. A similar immunologic analysis of a receptor-ligand interaction was used by Bruck *et al.* (19) to determine the sequences within reovirus that mediate binding to the reovirus receptor on mammalian cells. In this example, a sequence within the CDRs of an anti-idiotypic antibody reactive with the reovirus receptor was similar to a sequence within the receptor ligand, reovirus. Taken together with our results, these findings suggest that such an immunologic approach may be useful in the analysis of other cellular receptor-ligand interactions.

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[2] S. J. Shattil, J. Hoxie, R. Taub, and P. A. Friedman, unpublished observations.

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Specific Binding of a Synthetic Peptide Derived from an Antibody Complementarity Determining Region to Phosphatidylserine

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We have established a series of monoclonal antibodies that bind to phosphatidylserine (PS). One mAb, PS4A7, showed a strict specificity for PS and distinguished the stereospecific configuration of its serine moiety. We determined the amino acid sequences of the heavy and light chain variable regions of PS4A7, and examined the reactivity of the synthetic peptides corresponding to the complementarity determining region (CDR) of the mAb with phospholipids. We found that a 12-amino acid synthetic peptide corresponding to the third CDR of the heavy chain (amino acid residues 93-102, referred to as CDR3-H) bound specifically to PS. Although the affinity of the peptide to PS was markedly lower, the peptide was shown to bind to 1,2-diacyl-*sn*-glycero-3-phospho-L-serine (PS), but not to 1,2-diacyl-*sn*-glycero-3-phospho-D-serine, showing a similar specificity to that of PS4A7. The specific binding of the CDR3-H peptide to PS was confirmed by ELISA and TLC-immunostaining assay. The interaction between the CDR3-H peptide and water-soluble PS-derivatives was investigated by inhibition of the ELISA. PS effectively inhibited the binding and phosphoserine showed a weak but significant inhibition, but no appreciable inhibition was observed with serine. These observations suggest that the CDR3-H peptide plays a major role in the interaction of PS4A7 with the phosphoserine residue of the PS molecule.

Key words: anti-idiotypic antibody, anti-phospholipid antibody, complementarity determining region (CDR), monoclonal antibody, phosphatidylserine.

Phosphatidylserine (PS) has been shown to play various regulatory roles in biological responses (*1-3*). Several proteins have been shown to require PS for activation, and the structural requirement of PS is sometimes quite strict (*4-7*). Although PS may exhibit its biological activities through interacting with specific binding sites on these proteins, little information has been available about the protein structure which is responsible for the specific interaction with PS.

To elucidate the molecular mechanisms underlying the specific lipid-protein interactions, we have undertaken structural and idiotypic analyses of a monoclonal antibody, PS4A7, which binds specifically to PS (*8-10*). The combining sites of antibodies are formed by the juxtaposition of complementarity determining regions (CDRs), of which three are derived from the heavy chain (CDR1-, 2-, 3-H) and three from the light chain (CDR1-, 2-, 3-L). The CDRs form loops that protrude from the backbone barrel structure and these hypervariable loops construct the non-conserved antigen-binding sites (*11*). Several workers have demonstrated that synthetic peptides derived from the amino acid sequences of CDRs bound antigens with similar specificities to those of the original antibody molecules (*12-15*). These studies have prompted us to examine the functional role of the CDR fragments of the PS-specific mAb in the interaction with PS.

We have previously shown that the anti-idiotypic monoclonal antibody (anti-idiotypic mAb) raised against the combining site of PS4A7 cross-reacted extensively with protein kinase C (PKC) isoforms (α, β, γ) and inhibited the activation of the enzymatic activity (*10*). The binding of the anti-idiotypic mAb to PKC was inhibited specifically by PS, but not by other phospholipids including 1,2-diacyl-*sn*-glycero-3-phospho-D-serine and 1,2-diacyl-*sn*-glycero-3-phospho-L-homoserine. These observations suggest that the anti-idiotypic mAb recognizes a consensus structure between the PS-specific mAb and PKC, which is responsible for the specific interaction with PS.

In this study, we determined the amino acid sequences of the variable regions of PS4A7 to define the epitope recognized by the anti-idiotypic mAb, and synthesized a peptide which interacts with PS with similar specificity to that of PS4A7.

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Abbreviations: PS, phosphatidylserine; mAb, monoclonal antibody; CDR3-H, the third complementarity determining region of immunoglobulin heavy chain.

MATERIALS AND METHODS

Materials—PS from bovine brain white matter (*16, 17*), egg yolk phosphatidylcholine (PC) (*18*), *Escherichia coli* phosphatidylethanolamine (PE) (*19*), and bovine heart cardiolipin (CL) (*20*) were purified as previously described. Lysophosphatidylserine and alkylphosphoserine were prepared as described previously (*21*). Phosphatidic acid (PA) and phosphatidylinositol (PI) were purchased from Avanti Polar Lipids (Birmingham, AL). PS-specific mAb, PS4A7, was established as described previously (*8*) and an anti-idiotypic mAb, Id8F7, was established and characterized as described previously (*10*).

Sequencing of PS4A7 Variable Regions—N-terminal amino acid sequences and nucleotide sequences of the heavy and light chain variable regions of PS4A7 were determined by the method described previously (*22*). N-terminal sequences of heavy and light chains separated by HPLC gel filtration through a G3000SWXL (Tosoh, Tokyo) under reducing conditions were determined by automated Edman degradation using a protein sequencer (model 470A, Applied Biosystems). Oligonucleotide primers were synthesized by the triester method on a DNA synthesizer (model 381A, Applied Biosystems); the primers used were 5′-GCTCTCGCAGGAGACGAC-3′ for the heavy chain (μ primer) and 5′-GGGGCCAGTGGATAGAC-3′ for the light chain (κ primer). The mRNA from the hybridoma cells was annealed to the radiolabeled oligonucleotide primers and dideoxy sequencing was performed. The sequences thus obtained were confirmed by sequencing of cDNA amplified by the polymerase chain reaction (PCR). In brief, for the first strand cDNA synthesis, 75 μg total RNA was annealed to 100 ng of the μ or κ primer and extended with avian myeloblastosis virus (AMV) reverse transcriptase. For the amplification process, an aliquot of the cDNA first strand reaction mixture was supplemented with 100 pmol appropriate 3′ and 5′ primers with the *Eco*RI and *Bam*HI cloning sites and amplified in 100 μl of reaction mixture using 5 U Taq DNA polymerase, according to the manufacturer's protocol (Perkin Elmer Cetus). The cDNA corresponding to a band of 380–400 base pairs was extracted and ligated into M13mp19/18 phage vector. After transformation of the *E. coli* strain JM109, the integrated cDNA sequences were determined by the dideoxy sequencing method using a SEQUENASE sequencing kit (Toyobo, Osaka).

Binding of Id8F7 to Synthetic Peptides—Synthetic peptides (from 8 to 17 amino acids long) corresponding to the CDRs of PS4A7 were synthesized on 4-(oxymethyl)-phenylacetamidomethyl resins using an Applied Biosystems 430A automated peptide synthesizer. During the synthesis, an extra cysteine residue was added to the carboxyl terminus to couple the peptides to BSA. The synthetic peptides were purified by reverse-phase HPLC on an ODS120 column (Tosoh) and the sequences were confirmed using an Applied Biosystems 470A protein sequencer. The synthetic peptides were conjugated with BSA by the heterobifunctional cross-linking reagent maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) (Pierce Chemical, Rockford, IL) by a method of Lerner *et al.* (*23*). In brief, MBS was dissolved in dimethyl sulfoxide (1 mg/ml) and added to BSA solution (50 mM phosphate buffer, pH 8.0) to achieve a molar ratio of 40 : 1 of MBS : BSA. After stirring for 30 min, the free MBS was removed by gel filtration through a Sephadex G-25 column equilibrated with 50 mM phosphate buffer, pH 6.0. One milligram of synthetic peptides was added to the MBS activated BSA with stirring for 3 h and the free peptide was removed by gel filtration. The average molecular mass of the peptide-BSA complexes, which was determined by SDS PAGE, was about 90 kDa, indicating that an average of about 15 peptides were conjugated with BSA.

The peptide-BSA complexes were coated onto microtiter plates and the binding of Id8F7 to the synthetic peptides was examined by ELISA. In brief, microtiter plates were coated with 50 μl of the synthetic peptide-BSA conjugates [1 μg/ml in 10 mM Tris-HCl buffered saline (TBS), pH 7.4, overnight at 4°C], and were blocked by incubation with TBS containing 30 mg/ml BSA (3% BSA-TBS) for 2 h at room temperature. After washing with TBS, the plates were incubated with various amounts of a purified Id8F7 diluted with 1% BSA-TBS for 2 h at room temperature. The bound Id8F7 was detected with biotinylated anti-mouse immunoglobulin and peroxidase-conjugated streptavidin (Zymed Laboratories, San Francisco, CA).

Binding of the Biotinylated Peptides and Peptide-BSA Complexes to Phospholipids—BSA was labeled with biotin using *N*-hydroxysuccinimido biotin (Sigma) as previously described (*24*). The purified synthetic peptides were coupled through their cysteine residue to the biotin-labeled BSA using MBS as a cross-linker (Pierce). In addition, the peptides were biotinylated directly with iodoacetyl-LC-biotin (Pierce) using a slightly modified method of Sutoh *et al.* (*25*). In brief, the synthetic peptides (1 mg) in 10 mM Tris, 50 mM KCl, pH 8.5, were mixed with iodoacetyl-LC-biotin dissolved in dimethyl formamide at a molar ratio of 4 : 1 (iodoacetyl-LC-biotin : peptide). After incubation for 24 h at 4°C, the biotinylation reaction was quenched by addition of excess 2-mercaptoethanol and the biotin-labeled peptides were purified by reverse phase HPLC using an ODS120 column.

ELISA—The binding of the biotinylated peptides and peptide-BSA complexes to phospholipids was determined by ELISA as described previously (*20*). In brief, microtiter plate wells were coated with 50 μl of phospholipid antigen in ethanol (10 μM) by evaporation at room temperature. After the blocking of the wells with 10 mM HEPES, 150 mM NaCl, pH 7.4, containing 30 mg/ml BSA (3% BSA-HBS), the wells were incubated with various amounts of either the biotinylated peptides or peptide-BSA complexes. The relative fluorescence intensity was measured after the addition of 0.25 mM 4-methyl umbelliferyl phosphate substrate solution with a fluorescence ELISA reader (MTP-32, Corona Electric, Tokyo).

TLC-Immunostaining—The thin-layer chromatography (TLC) immunostaining assay was carried out using a slightly modified method of Karasawa *et al.* (*26*). Aliquots (10 and 50 nmol) of phospholipid antigen were spotted onto a high performance TLC plate (Art.5547, Merck, Darmstadt, Germany) and the plates were dipped into a chloroform/hexane (1 : 5) solution containing 0.4% (w/v) polyisobutylmethacrylate (Aldrich, Milwaukee, WI) for 5 s. After drying, the plates were soaked in TBS buffer for 5 min and then blocked with TBS containing 1% (w/v) ovalbumin and 1% (w/v) polyvinylpirrolidone (*M*

360,000) for 1.5 h. The plates were incubated with 10 μg of biotinylated peptide or peptide-BSA complex in TBS containing 1% (w/v) ovalbumin for 2 h. After three washings with TBS, the amounts of peptide or peptide-BSA complex bound to the phospholipid antigen were detected by adding peroxidase-conjugated streptavidin, followed by 4-chloro-1-naphthol solution (0.4 mg/ml, BioRad Laboratories, Richmond, CA) containing 0.01% H_2O_2 (*27*).

RESULTS AND DISCUSSION

Amino Acid Sequence of the PS-Specific mAb, PS4A7—In a previous study, we established a series of mAbs that bound to PS (*8*). One of them, named PS4A7, was highly specific to PS and distinguished the stereo-specific configuration of its serine moiety. To study the molecular mechanisms of the specific lipid-protein interaction, we first determined the amino acid sequences of the heavy and light chain variable regions of PS4A7. The mRNA sequences and the deduced amino acid sequences are shown in Fig. 1, A and B. The CDRs are indicated by solid lines and the sequences are numbered according to the scheme of Kabat *et al.* (*28*). The nucleotide sequences obtained indicate that the Vh, D, and J regions are encoded by the J558, S107.2.2, and Jh4 gene families, respectively, and the Vk and Jk regions are encoded by the Vk9 and Jk4 gene families. The most homologous Vk and Vh gene to that of PS4A7 was encoded by mAb L11-2E2 (anti-influenza virus hemagglutin, 96% homologous) (*29*) and mAb 126 (anti-insulin, 93% homologous) (*30*), respectively. None of the antibodies so far reported has the same combination of Vk and Vh gene segments as PS4A7.

Location of the Binding Site of Anti-Idiotypic mAb on PS4A7—In the previous study (*10*), we made a series of anti-idiotypic mAbs against the combining site of PS4A7. One of them, Id8F7, cross-reacted extensively with PKC, and its binding to PKC was specifically inhibited by PS, but not by synthetic PS analogs such as 1,2-diacyl-*sn*-glycero-3-phospho-D-serine and 1,2-diacyl-*sn*-glycero-3-phospho-L-homoserine. These results suggest that the anti-idiotypic mAb recognizes a consensus structure between the PS-specific mAb and PKC, which is responsible for the specific interaction with PS.

Although the combining sites of antibodies are formed by combination of the heavy and light chain CDRs, several studies have shown that the heavy chain CDRs play a major role in determining the antigen specificity of various mAbs (*31, 32*). To locate the Id8F7-binding site on PS4A7, we first synthesized the peptides corresponding to the CDRs of PS4A7 heavy chain and examined their reactivity with Id8F7. The synthetic peptides were coupled to BSA *via* a functional SH group at the carboxyl terminus and the binding of Id8F7 to the plate-coated peptide-BSA complex was determined by ELISA. Id8F7 bound specifically to the synthetic peptide derived from the third CDR of the PS4A7 heavy chain (AREGDYDGAMDY, amino acid residues 93–102, referred to as CDR3-H peptide), but not to the CDR2-H peptide (amino acid residues 50-65, LIMPYNGGTSYNQKFKG) or BSA conjugated with cysteine (Fig. 2). The CDR1-H peptide (FTGYTMKWV) tended to form aggregates and was unusable for the experiments.

The anti-idiotypic mAb effectively bound to the peptide

(A) V_H sequence of PS4A7

```
1                                                  10                                  20
 E  V  Q  L  Q  Q  S  G  P  E  L  V  K  P  G  A  S  M  K  I
GAG GTC CAG CTG CXA CAG TCT GGA CCT GAG CTG GTG AAG CCT GGA GCT TCA ATG AAG ATA
                                   30          CDR1                            40
 S  C  K  A  S  G  Y  S  F  T  G  Y  T  M  K  W  V  K  Q  S
TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC ACC ATG AAC TGG GTG AAG CAG AGC
                                   50          52A                 CDR2
 H  G  K  N  L  E  W  I  G  L  I  N  P  Y  N  G  G  T  S  Y
CAT GGA AAG AAC CTT GAG TGG ATT GGA CTT ATT AAT CCT TAC AAT GGT GGT ACT AGC TAC
60                                     70
 N  Q  K  F  K  G  K  A  T  L  T  V  D  K  S  S  S  T  A  Y
AAC CAG AAG TTC AAG GGC AAG GCC ACA TTA ACT GTA GAC AAG TCT TCC AGC ACA GCC TAC
80          82A 82B 82C                    90
 M  E  L  L  S  L  T  S  D  D  S  A  V  Y  Y  C  A  R  E  G
ATG GAG CTC CTC AGT CTG ACA TCT GAT GAC TCT GCA GTC TAT TAC TGT GCA AGA GAA GGG
          CDR3
 D  Y  D  G  A  M  D  Y  W  G  Q  G  T  S  V  T  V  S
GAT TAC GAC GGG GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
```

(B) V_L sequence of PS4A7

```
1                                                  10                                  20
 D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  L  G  G  K  V  T
GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT CTG GGA GGC AAA GTC ACC
          CDR1                30                                                  40
 I  T  C  K  S  S  Q  D  I  N  K  Y  I  A  W  Y  Q  H  K  P
ATC ACT TGC AAG TCA AGC CAA GAC ATT AAC AAG TAT ATA GCT TGG TAC CAA CAC AAG CCT
                                   50          CDR2                                60
 G  K  G  P  G  L  L  I  H  Y  T  S  T  L  Q  P  G  I  P  S
GGA AAA GGT CCT GGG CGT CTC ATA CAT TAC ACA TCT ACA TTA CAG CCA GGC ATC CCA TCA
                                   70                                  80
 R  F  S  G  S  G  S  G  R  D  Y  S  F  S  I  S  N  L  D  P
AGG TTC AGT GGA AGT GGG TCT GGG AGA GAT TAT TCC TTC AGC ATC AGC AAC CTG GAC CCT
                                   90          CDR3
 E  N  I  A  A  Y  Y  C  L  Q  Y  D  N  L  Y  T  F  G  G  G
GAA AAT ATT GCA GCT TAT TAT TGT CTA CAG TAT GAT AAT CTG TAC ACG TTC GGA GGG GXX

 T  R  L  E
ACC AGG CTG GAA
```

Fig. 1. **Nucleotide and deduced amino acid sequences of the heavy and light chain variable regions of the anti-PS mAb PS4A7.** The nucleotide sequences of the heavy (A) and light (B) chains were determined and the deduced amino acid sequences are shown above the sequences. The CDRs defined by Kabat *et al.* (*28*) are indicated by solid lines (—).

which had been coupled to BSA, but not appreciably to the free peptide directly coated onto the plates (data not shown). The observation suggests that Id8F7 recognizes a conformational structure of the CDR3-H peptide, which may be altered when the peptide is coated directly onto the solid surface.

Binding of the CDR3-H Peptide to PS—To study the functional role of the CDR3-H sequence in binding of the antibody molecule to PS, we analyzed the interaction between the CDR3-H peptide and various phospholipids. The CDR3-H peptide and the CDR3-H peptide without arginine residue [EGDYDGAMDY, referred to as CDR3-H(R−)] were conjugated with biotin-labeled BSA *via* the free cysteine residue at the C-terminus [CDR3-H and CDR3-H(R−) complex] and the binding of the peptides to various phospholipids was assessed by an ELISA and a TLC-immunostaining assay.

In ELISA, the CDR3-H complex bound effectively to PS, while CDR3-H(R−) complex did not show any significant binding to the phospholipids examined in this study (Fig. 3). The result indicates that the CDR3-H peptide could interact with PS, and that its arginine residue is essential for the interaction. Neither CDR1-H complex nor CDR2-H complex bound to PS (data not shown). The interaction between the CDR3-H complex and PS was highly specific and the complex did not bind appreciably to a synthetic PS analog, 1,2-diacyl-*sn*-glycero-3-phospho-D-serine (Fig. 4). The reactivity of the CDR3-H complex with the "soluble" compounds was evaluated by inhibition of the ELISA (Fig. 5). Small unilamellar vesicles of PS markedly inhibited the

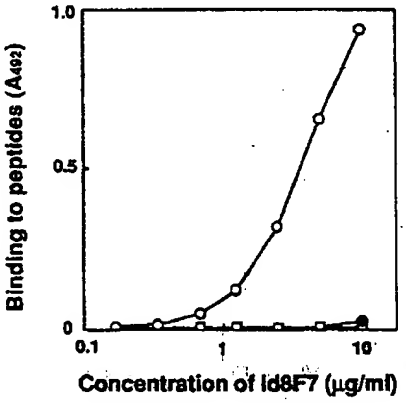

Fig. 2. **The binding of Id8F7 to the synthetic peptides.** The synthetic peptides derived from the heavy chain of PS4A7, CDR3-H [amino acid residues 93-102, AREDGYDGAMDY] (○), CDR2-H [amino acid residues 50-65, LIMPYNGGTSYNQKFKG] (●), and cysteine as a control (□) were coupled to BSA *via* the functional SH group at the carboxyl terminus. Peptide-BSA complexes (50 ng/well) were coated on microtiter wells and the binding of Id8F7 to each peptide was examined. Id8F7 bound to the peptide-BSA complexes was detected with biotinylated anti-mouse IgM and peroxidase-conjugated streptavidin.

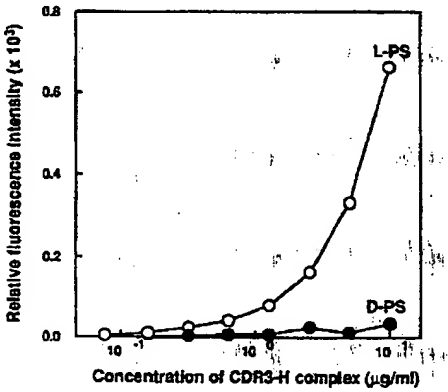

Fig. 4. **Specific binding of the CDR3-H peptide to PS.** The wells of the microtiter plate were coated with either 1,2-diacyl-*sn*-glycero-3-phospho-L-serine (L-PS; ○) or 1,2-diacyl-*sn*-glycero-3-phospho-D-serine (D-PS; ●) (10 μM). The binding of the CDR3-H-BSA complex to the plate-coated phospholipids was examined by ELISA as described in Fig. 3.

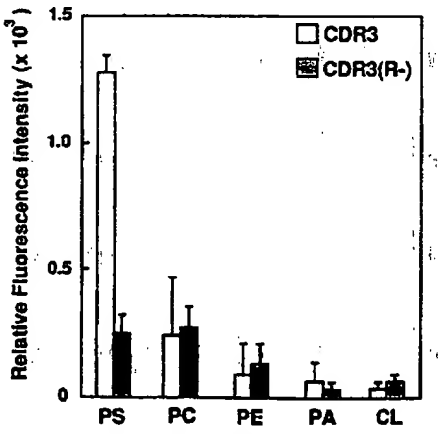

Fig. 3. **Binding of the peptide-BSA complexes to the phospholipids.** The CDR3-H and CDR3-H (R−) were coupled to biotinylated BSA and the resulting peptide-BSA complexes (5 μg/ml) were incubated with various phospholipids (10 μM) (PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; CL, cardiolipin) coated onto microtiter plate wells. The complexes bound were detected with alkaline phosphatase-conjugated streptavidin. Each bar represents the mean value ± SD of three different experiments.

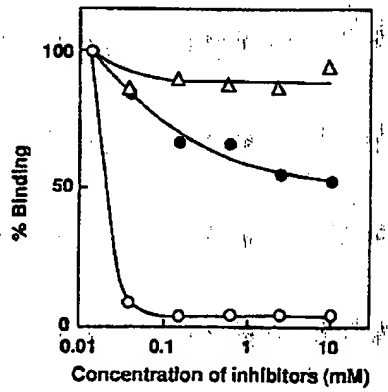

Fig. 5. **Inhibition of the binding of CDR3-H peptide-BSA by soluble haptens.** The CDR3-H peptide-BSA complex (50 ng) was preincubated with PS (○), phosphoserine (●), or serine (△). The mixtures were transferred to the PS-coated microtiter plate wells and the CDR3-H peptide-BSA complex bound was detected with peroxidase-conjugated streptavidin.

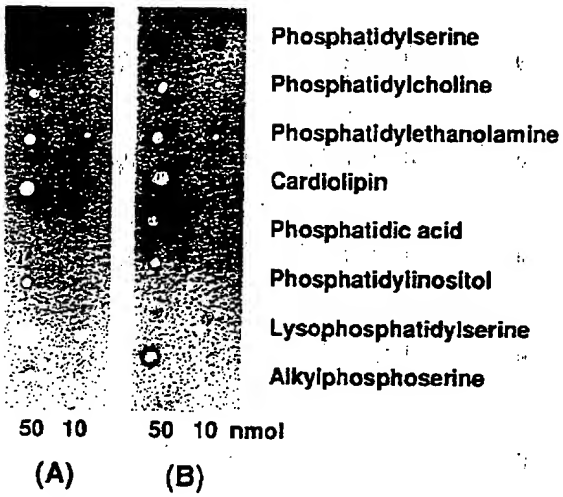

Fig. 6. **TLC-immunostaining analysis of the binding of CDR3-H peptide to phospholipids.** Various phospholipids (10 and 50 nmol) were spotted onto a HPTLC plate and incubated with the biotinylated CDR3-H peptide (A) or CDR3-H peptide-BSA complex (B). The peptides bound were detected with streptavidin conjugated peroxidase.

binding, and phosphoserine showed a weak but significant inhibition, but no appreciable inhibition was observed with serine. Fifty percent inhibition of the binding of CDR3-H complex was observed with 0.01 mM PS and 1.25 mM phosphoserine. In our previous study, water-soluble derivatives, such as phosphoserine and glycero-phosphoserine, did not inhibit the binding of PS4A7 (*8*). These observations suggest that the CDR3-H peptide plays a major role in the interaction with the phosphoserine residue of the PS molecule and that the secondary interactions between PS4A7 and the hydrophobic portion of the antigen molecule may affect the stability of the antibody-antigen complex, resulting in the higher affinity binding.

We also examined the binding to PS of the free CDR3-H peptide, which had been directly biotinylated with iodoacetyl-LC-biotin at the cysteine residue of the C-terminus. However, no significant binding was observed (data not shown). The average molecular mass of the CDR3-H-BSA complex, which was determined by SDS-PAGE, was about 90 kDa, indicating that an average of about 15 peptides were conjugated with BSA. It is likely that the multivalent interaction of the CDR3-H complex with PS significantly enhances the avidity of the interaction.

In contrast to the ELISA, significant binding of the free CDR3-H peptide to PS was observed in the TLC-immunostaining assay. Both the free CDR3-H peptide and the peptide-BSA complex were shown to bind specifically to PS and cross-reacted weakly with lysophosphatidylserine, alkylphosphoserine, cardiolipin, and phosphatidic acid (Fig. 6). Our preliminary analysis using isotope-labeled phospholipids showed that with the ELISA approximately 0.1 nmol plate-coated phospholipids (20% of those present in the assay system) remained on the plate surface throughout the assay procedure, whereas with the TLC-immunostaining assay almost all phospholipids (more than 10 nmol per dot) remained on the plates, therefore there was a greater than 100-fold difference between the antigen densities on the solid phases. Although we did not measure the affinity of the peptide for PS precisely, a cross-competition analysis using the ELISA showed that the order of the ligand-binding avidities to PS was PS4A7≫BSA-conjugated peptide>free peptide (unpublished observations). As a result of the low antigen density on the wells of the microtiter plates and the extensive washing procedures used in the ELISA, the TLC-immunostaining assay appears to be more sensitive than the ELISA for measuring the binding of low affinity ligands, such as free CDR3-H peptide, to phospholipids.

The present analyses showed that the anti-idiotypic mAb Id8F7, which was shown to recognize a consensus PS-binding structure between the PS-specific mAb PS4A7 and PKC (*10*), bound specifically to the synthetic peptide derived from the CDR3 of PS4A7 heavy chain. The synthetic peptide was also shown to bind specifically to PS, indicating that the peptide sequence may represent a novel PS-recognizing peptide motif of the mAb. The peptide will provide a useful structural model to study the molecular mechanisms of the specific PS-protein interaction and a basic structural template to develop a high affinity PS-binding peptide to probe the molecular motion of PS in biological membranes.

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United States Court of Appeals,
Federal Circuit.

ENZO BIOCHEM, INC., Plaintiff-Appellant,
v.
GEN-PROBE INCORPORATED,
and
Chugai Pharma U.S.A., Inc. and Chugai Pharmaceutical Co., Ltd.,
and
Biomerieux, Inc.,
and
Becton Dickinson and Company, Defendants-Appellees,
and
Biomerieux SA, Defendant.

No. 01-1230.

DECIDED: July 15, 2002.

Assignee of patent directed to nucleic acid probes that selectively hybridize to genetic material of bacteria that cause gonorrhea brought patent infringement suit against competitors, who moved for summary judgment. The United States District Court for the Southern District of New York, Alvin K. Hellerstein, J., granted motion. Assignee appealed. On grant of petition for rehearing, the Court of Appeals, Lourie, Circuit Judge, held that: (1) patent's reference to deposit in public depository can constitute adequate description of claimed material for purpose of written description requirement; (2) fact issues existed as to whether one skilled in the art would view various subsequences, mutations, and mixtures of deposited sequences as within scope of claims; (3) fact issues existed as to whether deposited sequences were representative of broader genus claims; (4) fact issues existed as to whether claimed sequences were adequately described in terms of function; and (5) specification's indication that assignee possessed claimed invention by reducing it to practice was insufficient alone to meet written description requirement.

Reversed and remanded.

Petition for rehearing en banc denied.

Lourie, Circuit Judge, filed an opinion concurring in denial of petition for rehearing en banc, in which Pauline Newman, Circuit Judge, joined.

Pauline Newman, Circuit Judge, filed an opinion concurring in denial of rehearing en banc.

Dyk, Circuit Judge, filed an opinion concurring in denial of rehearing en banc.

Rader, Circuit Judge, filed an opinion dissenting from denial of rehearing en banc, in which Gajarsa and Linn, Circuit Judges, joined.

Opinion, 285 F.3d 1013, vacated.

West Headnotes

[1] Patents 112.5
291k112.5 Most Cited Cases

A patent is presumed to be valid, and this presumption can be overcome only by facts supported by clear and convincing evidence to the contrary. 35 U.S.C.A. § 282.

[2] Patents 314(5)
291k314(5) Most Cited Cases

Compliance with the patent statute's written description requirement is a question of fact. 35 U.S.C.A. § 112.

[3] Patents 99
291k99 Most Cited Cases

Written description requirement of patent statute calls for a written description of an invention separate from enablement. 35 U.S.C.A. § 112.

[4] Patents 99
291k99 Most Cited Cases

Compliance with the patent statute's written description requirement is essentially a fact-based inquiry that will necessarily vary depending on the nature of the invention claimed. 35 U.S.C.A. § 112.

[5] Patents 97
291k97 Most Cited Cases

Regulatory guidelines governing internal practice of Patent and Trademark Office (PTO) for examining patent applications under statutory written description requirement, like the Manual of Patent Examining Procedure (MPEP), are not binding on Court of Appeals, but may be given judicial notice to the

extent they do not conflict with the statute. 35 U.S.C.A. § 112.

[6] Patents 99
291k99 Most Cited Cases

Patent statute's written description requirement can be met by showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. 35 U.S.C.A. § 112.

[7] Patents 99
291k99 Most Cited Cases

Reference in patent specification to deposits of claimed nucleotide sequences in public depository sufficiently described those sequences to the public for purposes of patent statute's written description requirement; a person of skill in the art, reading the accession numbers in the patent specification, could obtain claimed sequences from depository by following the appropriate techniques to excise the nucleotide sequences from the deposited organisms containing those sequences, and, although structures of those sequences were not expressly set forth in the specification, those structures may not have been reasonably obtainable and in any event were not known to patent applicant when application was filed. 35 U.S.C.A. § 112.

[8] Patents 99
291k99 Most Cited Cases

Reference in patent specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the patent statute's written description requirement. 35 U.S.C.A. § 112.

[9] Federal Civil Procedure 2508
170Ak2508 Most Cited Cases

Genuine issue of material fact as to whether three claimed nucleotide sequences placed in public depository also described various subsequences, mutations, and mixtures of those sequences, which allegedly also fell within scope of patent claims, to one skilled in the art precluded summary judgment for alleged infringer, who asserted that such substantial breadth of claims would render them invalid under the written description requirement. 35 U.S.C.A. § 112.

[10] Federal Civil Procedure 2508
170Ak2508 Most Cited Cases

Genuine issue of material fact as to whether three claimed nucleotide sequences placed in public depository were representative, to one skilled in the art, of broad genus claims, in patent directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, precluded summary judgment for alleged infringer on its claim that genus claims were invalid for failure to meet patent statute's written description requirement. 35 U.S.C.A. § 112.

[11] Federal Civil Procedure 2508
170Ak2508 Most Cited Cases

Genuine issue of material fact as to whether disclosed correlation of their function of hybridization with bacterial DNA strains deposited in public depository described claimed nucleotide sequences, in patent directed to nucleic acid probes that selectively hybridized to genetic material of bacteria that caused gonorrhea, even though DNA structures were not explicitly sequenced, precluded summary judgment for alleged infringer on its claim that patent was invalid for failure to meet the written description requirement. 35 U.S.C.A. § 112.

[12] Patents 99
291k99 Most Cited Cases

Fact that claims of patent directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea appeared in ipsis verbis in the written description did not automatically satisfy written description requirement of patent statute. 35 U.S.C.A. § 112.

[13] Patents 99
291k99 Most Cited Cases

Even if a patent claim is supported by the specification, the language of the specification, to the extent possible, must describe the claimed invention so that one skilled in the art can recognize what is claimed; the appearance of mere indistinct words in a specification or a claim, even an original claim,

does not necessarily satisfy that requirement. 35 U.S.C.A. § 112.

[14] Patents 99
291k99 Most Cited Cases

A description of what a material does, rather than of what it is, usually does not suffice to meet the patent statute's written description requirement; disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. 35 U.S.C.A. § 112.

[15] Patents 99
291k99 Most Cited Cases

Where the words of the patent claim alone do not convey an adequate description of the invention, regardless of whether the claim appears in the original specification and is thus supported by the specification as of the filing date, the patent statute's written description requirement is not necessarily met. 35 U.S.C.A. § 112.

[16] Patents 99
291k99 Most Cited Cases

If a purported description of an invention does not meet patent statute's written description requirement, the fact that it appears as an original claim or in the specification does not save it; a claim does not become more descriptive by its repetition, or its longevity. 35 U.S.C.A. § 112.

[17] Patents 99
291k99 Most Cited Cases

Mere fact that specification of patent, which was directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, indicated that patent holder possessed claimed invention by reducing to practice three nucleotide sequences within the scope of the patent claims and depositing them in public depository did not establish that patent met the statutory written description requirement. 35 U.S.C.A. § 112.

[18] Patents 99
291k99 Most Cited Cases

While articulation of written description requirement of patent statute in terms of "possession" is useful when a patentee is claiming entitlement to an earlier filing date, in interferences in which the issue is whether a count is supported by the specification of one or more of the parties, and in ex parte applications in which a claim at issue was filed subsequent to the application, application of written description requirement is not subsumed by the "possession" inquiry. 35 U.S.C.A. § 112.

[19] Patents 99
291k99 Most Cited Cases

A showing that the patentee is in "possession" of the claimed invention is ancillary to the statutory mandate of an adequate written description, and that requirement is not met if, despite a showing of possession, the specification does not adequately describe the claimed invention. 35 U.S.C.A. § 112.

[20] Patents 99
291k99 Most Cited Cases

Although one can show possession of an invention by means of an affidavit or declaration during prosecution, as one does in an interference or when one files an affidavit to antedate a reference, such a showing of possession does not substitute for a written description in the specification, as required by patent statute. 35 U.S.C.A. § 112.

[21] Patents 99
291k99 Most Cited Cases

Proof of a reduction to practice, absent an adequate description in the specification of what is reduced to practice, does not serve to describe or identify the invention for purposes of the patent statute's written description requirement. 35 U.S.C.A. § 112.

[22] Patents 99
291k99 Most Cited Cases

Written description requirement is the quid pro quo of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time. 35 U.S.C.A. § 112.

***959** Richard L. Delucia, Kenyon & Kenyon, of New York, NY, filed a petition for rehearing en banc for plaintiff-appellant. With him on the petition were Charles A. Weiss and Bradley S. Corsello.

The appellees filed a consolidated response to the petition for rehearing en banc. William F. Lee, Hale and Dorr LLP, of Boston, MA, for defendant-

appellee **Gen-Probe** Incorporated. With him on the response was William G. McElwain.

Robert J. Gunther, Jr., Latham & Watkins, of New York, NY, for defendants- ***960** appellees Chugai Pharma U.S.A., Inc. and Chugai Pharmaceutical Co., Ltd. With him on the response was Jeffrey A. Tochner. Of counsel was Kurt M. Rogers.

Daniel A. Boehnen, McDonnell Boehnen Hulbert & Berghoff, of Chicago, IL, for defendant-appellee Biomerieux, Inc. With him on the response was Joshua R. Rich.

Donald R. Ware, Foley Hoag & Eliot LLP, of Boston, MA, for defendant- appellee Becton Dickinson and Company. With him on the response was Barbara A. Fiacco.

Frank P. Porcelli, Fish & Richardson P.C., of Boston, MA, filed a brief for amicus curiae Fish & Richardson P.C. Of counsel on the brief were Robert E. Hillman and Charles H. Sanders.

Mark S. Davies, Attorney, Appellate Staff, Civil Division, Department of Justice, of Washington, DC, filed an amicus curiae brief for the United States in support of rehearing en banc. With him on the brief were Robert D. McCallum, Jr., Assistant Attorney General, and Scott R. McIntosh, Attorney. Of counsel on the brief was John M. Whealan, Solicitor, U.S. Patent and Trademark Office, of Arlington, Virginia.

Before LOURIE, DYK and PROST, Circuit Judges.

ON PETITION FOR REHEARING

LOURIE, Circuit Judge.

Enzo Biochem, Inc. petitions for rehearing of this appeal following our prior decision, reported at 285 F.3d 1013, 62 USPQ2d 1289 (Fed.Cir.2002), in which we affirmed the decision of the United States District Court for the Southern District of New York. The district court had granted **Gen-Probe** Incorporated, Chugai Pharma U.S.A., Inc., Chugai Pharmaceutical Co., Ltd., Biomerieux, Inc., Biomerieux SA, and Becton Dickinson and Company's (collectively, "the defendants") motion for summary judgment that claims 1-6 of U.S. Patent 4,900,659 are invalid for failure to meet the written description requirement of 35 U.S.C. § 112, ¶ 1. *Enzo Biochem, Inc. v. **Gen-Probe** Inc.*, No. 99 Civ. 4548 (S.D.N.Y. Apr. 4, 2001) (final order). Having considered Enzo's petition for rehearing and the defendants' response, [FN1] we have determined that our prior decision that a deposit may not satisfy the written description requirement was incorrect. We therefore grant Enzo's petition for rehearing, vacate the prior decision, and reverse the district court's grant of summary judgment that Enzo's claims are invalid for failure to meet the written description requirement. Because genuine issues of material fact exist regarding satisfaction of the written description requirement, we remand.

FN1. *Amicus curiae* briefs were filed by the United States Patent and Trademark Office and Fish & Richardson P.C.

BACKGROUND

Enzo is the assignee of the '659 patent, which is directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, *Neisseria gonorrhoeae*. *N. gonorrhoeae* reportedly has between eighty and ninety-three percent homology with *Neisseria meningitidis*. '659 patent, col. 2, ll. 61-64. Such a high degree of homology has made detection of *N. gonorrhoeae* difficult, as any probe capable of detecting *N. gonorrhoeae* may also show a positive result when only *N. meningitidis* is present. Enzo recognized the need for a chromosomal DNA probe specific for *N. **961** gonorrhoeae*, and it derived three such sequences that preferentially hybridized to six common strains of *N. gonorrhoeae* over six common strains of *N. meningitidis*. Id. at col. 3, l. 49 to col. 4, l. 14; col. 4, ll. 45-50. The inventors believed that if the preferential hybridization ratio of *N. gonorrhoeae* to *N. meningitidis* were greater than about five to one, then the "discrete nucleotide sequence [would] hybridize to virtually all strains of *Neisseria gonorrhoeae* and to no strain of *Neisseria meningitidis*." Id. at col. 12, ll. 60-65. The three sequences that the inventors actually derived had a selective hybridization ratio of greater than fifty. Id. at col. 13, ll. 9-15. Enzo deposited those sequences in the form of a recombinant DNA molecule within an *E. coli* bacterial host at the American Type Culture Collection. Id. at col. 13, ll. 27-31.

Claim 1 is as follows:

1. A composition of matter that is specific for *Neisseria gonorrhoeae* comprising at least one nucleotide sequence *for which the ratio of the*

amount of said sequence which hybridizes to chromosomal DNA of Neisseria gonorrhoeae to the amount of said sequence which hybridizes to chromosomal DNA of Neisseria meningitidis is greater than about five, said ratio being obtained by a method comprising the following steps;

(a) providing a radioactively labeled form of said nucleotide sequence;

(b) providing a serial dilution series of purified chromosomal DNA from each of the *N. gonorrhoeae* strains; (1) ATCC 53420, (2) ATCC 53421, (3) ATCC 53422, (4) ATCC 53423, (5) ATCC 53424, (6) ATCC 53425, and forming test dots from each of said dilution series on a matrix;

(c) providing a serial dilution series of purified nucleotide sequences from each of the *N. meningitidis* strains: (1) ATCC 53414, (2) ATCC 53415, (3) ATCC 53416, (4) ATCC 53417, (5) ATCC 53418, (6) ATCC 53419, and forming test dots from each of said dilution series on a matrix;

(d) hybridizing equal portions of the labeled nucleotide sequences to the matrix provided in step (b) and (c), respectively; wherein the hybridization is conducted in a solution having a salt concentration of 2X SSC at (i) 65° C. in cases in which the sequence has greater than 50 base pairs or (ii) at Tm (° C.) minus 30° C. in cases in which the sequence has less than 50 base pairs, wherein Tm is the denaturation temperature of the sequence;

(e) quantifying the labeled nucleotide sequence hybridized in step (d) to each test dot;

(f) subtracting from the data of step

(e) an averaged amount of radioactivity attributable to background to obtain a corrected amount of hybridized radioactivity at each test dot;

(g) normalizing the data of step (f) by multiplying the amount of corrected radioactivity at each test dot by a factor which adjusts the amount of radioactivity to equal amounts of chromosomal DNA at each test dot;

(h) selecting two normalized values that are most nearly the same and that correspond to adjacent members of the dilution series for each of the above strains of *N. gonorrhoeae* and obtaining the average of the selected values;

(i) selecting two normalized values that are most nearly the same and that correspond to adjacent members of the dilution series for each of the above strains of *N. meningitidis* and ***962** obtaining the average of the selected values;

(j) dividing the lowest average obtained in step (h) by the highest average obtained in step (i) to obtain said ratio.

Id. at col. 27, l. 29 to col. 28, l. 27 (emphasis added). Claims 2 and 3 depend from claim 1 and further limit the hybridization ratio to greater than about twenty-five and fifty, respectively. Id. at col. 2, ll. 27-30. Claim 4 is directed to the three deposited sequences (referenced by their accession numbers) and variants thereof as follows:

> 4. The composition of claim 1 wherein said nucleotide sequences are selected from the group consisting of:
>
> a. the *Neisseria gonorroheae* [sic] DNA insert of ATCC 53409, ATCC 53410 and ATCC 53411, and discrete nucleotide subsequences thereof,
>
> b. mutated discrete nucleotide sequences of any of the foregoing inserts that are within said hybridization ratio and subsequences thereof; and
>
> c. mixtures thereof.

Id. at col. 28, ll. 31-39. Claim 5 is directed to an assay for detection of *N. gonorrhoeae* using the composition of claim 1. Id. at ll. 40-46. Claim 6 further limits the method of claim 5 to the nucleotide sequences that Enzo deposited (*i.e.,* those in claim 4) and variants thereof. Id. at ll. 47-56.

Enzo sued the defendants for infringement of the '659 patent, and the defendants moved for summary judgment that the claims were invalid for failure to meet the written description requirement of 35 U.S.C. § 112, ¶ 1. The district court, in oral remarks from the bench, granted that motion. Tr. of Hr'g at 42, *Enzo Biochem, Inc. v.* ***Gen-Probe,*** *Inc.*, No. 99-CV-4548 (S.D.N.Y. Jan. 24, 2001). It concluded that the claimed composition of matter was defined only by its biological activity or function, *viz.,* the ability to hybridize to *N. gonorrhoeae* in a ratio of better than about five with respect to *N. meningitidis,* which it was held was insufficient to satisfy the § 112, ¶ 1 requirement set forth in this court's holdings in *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed.Cir.1997), *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed.Cir.1993), and *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed.Cir.1991). Tr. of Hr'g at 28. The court rejected Enzo's argument that the reference in the specification to the deposits of biological materials in a public depository inherently disclosed that the inventors were in possession of the claimed sequences. *Id.* at 35. It distinguished this court's precedents concerning deposits as relating to the enablement requirement of § 112, ¶ 1. *Id.* at 38-40. Enzo appealed to this court; we have jurisdiction pursuant to 28 U.S.C. § 1295(a)(1).

DISCUSSION

[1][2] Summary judgment is appropriate when there is no genuine issue of material fact and the moving party is entitled to judgment as a matter of law. Fed.R.Civ.P. 56(c); *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 247-48, 106 S.Ct. 2505, 91 L.Ed.2d 202 (1986). On motion for summary judgment, the court views the evidence and any disputed factual issues in the light most favorable to the party opposing the motion. *Matsushita Elec. Indus. Co. v. Zenith Radio Corp.*, 475 U.S. 574, 587, 106 S.Ct. 1348, 89 L.Ed.2d 538 (1986). A patent is presumed to be valid, 35 U.S.C. § 282 (1994), and this presumption can be overcome only by facts supported by clear and convincing evidence to the contrary, *see, e.g., WMS Gaming, Inc. v. Int'l Game Tech.*, 184 F.3d 1339, 1355, 51 USPQ2d 1385, 1396-97 (Fed.Cir.1999). Compliance with the written description ***963** requirement is a question of fact. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed.Cir.1991).

Enzo argues that the testimony of its expert, Dr. Wetmer, raised a genuine factual issue whether the reference to the deposits inherently described the claimed nucleotide sequences. Enzo also argues that its description of the binding affinity of the claimed nucleotide sequences satisfies the requirement set forth in the Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, 66 Fed. Reg. 1099 (Jan. 5, 2001) ("*Guidelines*"). Enzo asserts that the court erred in not evaluating the patentability of the claims separately, pointing out that claims 4 and 6 are directed to the three deposited sequences and variations and mixtures thereof. Enzo further asserts that the claims *per se* meet the written description requirement because they appear *in ipsis verbis* in the written description. Enzo also argues that this court's articulation of the written description requirement for genetic material in *Eli Lilly* should not apply to this case because Enzo reduced the invention to practice and deposited the derived biological materials, thereby demonstrating its "possession" of the invention.

The defendants respond that the district court properly granted summary judgment because the patent described the claimed nucleotide sequences only by their function, which they state is insufficient to meet the requirements of § 112, ¶ 1 as a matter of law, even as to the narrower claims directed to the deposited materials. The defendants also assert that Dr. Wetmur's opinion that the deposited genetic materials could have been sequenced did not cure the actual failure of the inventors to identify them by some distinguishing characteristic, such as their structure. Moreover, the defendants point out that claims 4 and 6, which are directed to the deposited materials, each cover a broad genus of nucleic acids. The defendants also urge that *in ipsis verbis* support for the claims in the specification does not *per se* establish compliance with the written description requirement. Finally, the defendants assert that the district court did not err in its determination that Enzo's "possession" of three nucleotide sequences that it reduced to practice and deposited nevertheless did not satisfy the written description requirement of § 112, ¶ 1.

[3][4] The written description requirement of § 112, ¶ 1 is set forth as follows:

> *The specification shall contain a written description of the invention,* and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 U.S.C. § 112, ¶ 1 (1994) (emphasis added). We have interpreted that section as requiring a "written description" of an invention separate from enablement. *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1117 (recognizing the severability of the "written description" and "enablement" provisions of § 112, ¶ 1). Compliance with the written description requirement is essentially a fact-based inquiry that will "necessarily vary depending on the nature of the invention claimed." *Id.* (citing *In re DiLeone*, 58 C.C.P.A. 925, 436 F.2d 1404, 1405, 168 USPQ 592, 593 (1971)). We have also previously considered the written description requirement as applied to certain biotechnology patents, in which a gene material has been defined only by a statement of function or result, and have held that ***964** such a statement alone did not adequately describe the claimed invention. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. In *Eli Lilly*, we concluded that a claim to a microorganism containing a human insulin cDNA was not adequately described by a statement that the invention included human insulin cDNA. *Id.* at 1567, 43 USPQ2d at 1405. The recitation of the term human insulin cDNA conveyed no distinguishing information about the identity of the claimed DNA sequence, such as its relevant structural or physical characteristics. *Id.* We stated that an adequate written description of genetic material " 'requires a precise definition, such as by structure, formula, chemical name, or physical

properties,' not a mere wish or plan for obtaining the claimed chemical invention," and that none of those descriptions appeared in that patent. *Id.* at 1566, 43 USPQ2d at 1404 (quoting *Fiers*, 984 F.2d at 1171, 25 USPQ2d at 1606). The specification in the *Eli Lilly* case thus did not show that the inventors had possession of human insulin cDNA.

[5][6] It is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement. The PTO has issued Guidelines governing its internal practice for addressing that issue. The Guidelines, like the Manual of Patent Examining Procedure ("MPEP"), are not binding on this court, but may be given judicial notice to the extent they do not conflict with the statute. *See Molins PLC v. Textron, Inc.*, 48 F.3d 1172, 1180 n. 10, 33 USPQ2d 1823, 1828 n. 10 (Fed.Cir.1995). In its Guidelines, the PTO has determined that the written description requirement can be met by "show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics ... *i.e.*, complete or partial structure, other physical and/or chemical properties, *functional characteristics when coupled with a known or disclosed correlation between function and structure,* or some combination of such characteristics." *Guidelines*, 66 Fed. Reg. at 1106 (emphasis added). For example, the PTO would find compliance with 112, 1, for a claim to an isolated antibody capable of binding to antigen X, notwithstanding the functional definition of the antibody, in light of the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature. Synopsis of Application of Written Description Guidelines, at 60, *available at* http://www.uspto.gov/web/patents/guides.htm (*Application of Guidelines*). Thus, under the Guidelines, the written description requirement would be met for all of the claims of the '659 patent if the functional characteristic of preferential binding to *N. gonorrhoeae* over *N. meningitidis* were coupled with a disclosed correlation between that function and a structure that is sufficiently known or disclosed. We are persuaded by the Guidelines on this point and adopt the PTO's applicable standard for determining compliance with the written description requirement.

Applying those principles, we first inquire whether Enzo's deposits of the claimed nucleotide sequences of claims 4 and 6 may constitute an adequate description of those sequences. Secondly, we will consider whether the description requirement is met for all of the claims on the basis of the functional ability of the claimed nucleotide sequences to hybridize to strains of *N. gonorrhoeae* that are accessible by deposit.

[7][8] As to the first question, Enzo asserts that the claimed sequences are inherently described by reference to deposits of three sequences that are within the ***965** scope of its claims. Whether reference to a deposit of a nucleotide sequence may adequately describe that sequence is an issue of first impression in this court. In light of the history of biological deposits for patent purposes, the goals of the patent law, and the practical difficulties of describing unique biological materials in a written description, we hold that reference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the written description requirement of § 112, ¶ 1.

The practice of depositing biological material arose primarily to satisfy the enablement requirement of § 112, ¶ 1. For example, in *In re Argoudelis*, the patent application claimed antibiotic compounds that were produced by a microorganism. 58 C.C.P.A. 769, 434 F.2d 1390, 1390, 168 USPQ 99, 100 (1970). The applicants deposited the microorganism because they could not "sufficiently disclose by written word how to obtain the microorganism starting material from nature." *Id.* at 1392, 168 USPQ at 102. By making the biological material accessible to the public, they enabled the public to make and use the claimed antibiotics. *Id.* at 1393, 168 USPQ at 102-03. In *Amgen*, we noted the relevance of deposit practice to satisfaction of the enablement requirement but rejected the defendants' argument that a deposit was necessary in that case to satisfy the best mode requirement of § 112, ¶ 1. *See* 927 F.2d at 1210, 18 USPQ2d at 1024; *see also In re Lundak*, 773 F.2d 1216, 1217, 227 USPQ 90, 92 (Fed.Cir.1985) (discussing deposit practice primarily in relation to an enablement rejection and noting that "[a]n accession number and deposit date add nothing to the written description of the invention" in the context of proven availability of a cell line prior to filing date).

Recognizing the importance of biological deposits to patent practice, the PTO has promulgated rules to address the procedural requirements relating to such deposits, but it has declined to expressly correlate substantive requirements relating to deposits with particular statutory requirements. *See* Deposit of

Biological Materials for Patent Purposes, 53 Fed. Reg. 39,420, 39,425 (Oct. 6, 1988) (notice of proposed rules) (codified at 37 C.F.R. Part 1) ("The rules are not intended to address which requirements of 35 U.S.C. 112 may be met by the making of deposits."). The Office does offer guidance, however, in determining when a deposit may be necessary, such as "[w]here the invention involves a biological material and words alone cannot sufficiently describe how to make and use the invention in a reproducible manner." MPEP § 2402 (8th ed. Aug. 2001). The PTO has also issued a regulation stating when a deposit is not necessary, *i.e.,* "if it is known and readily available to the public or can be made or isolated without undue experimentation." 37 C.F.R. § 1.802(b) (2001). Inventions that cannot reasonably be enabled by a description in written form in the specification, but that otherwise meet the requirements for patent protection, may be described in surrogate form by a deposit that is incorporated by reference into the specification. While deposit in a public depository most often has pertained to satisfaction of the enablement requirement, we have concluded that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material.

In this case, Enzo's deposits were incorporated by reference in the specification. A person of skill in the art, reading the ***966** accession numbers in the patent specification, can obtain the claimed sequences from the ATCC depository by following the appropriate techniques to excise the nucleotide sequences from the deposited organisms containing those sequences. '659 patent, col. 13, ll. 27-36. The sequences are thus accessible from the disclosure in the specification. Although the structures of those sequences, *i.e.,* the exact nucleotide base pairs, are not expressly set forth in the specification, those structures may not have been reasonably obtainable and in any event were not known to Enzo when it filed its application in 1986. *See* '659 patent, col. 3, ll. 40-46 (noting severe time constraints in sequencing DNA). We therefore agree with Enzo that reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement.

[9] As the defendants point out, however, Enzo's claims 4 and 6 are not limited to the deposited sequences. Claim 4 is directed to nucleotide sequences that are selected from the group consisting of the three deposited sequences, "discrete nucleotide subsequences thereof ... mutated discrete nucleotide sequences of any of the foregoing inserts that are within said hybridization ratio and subsequences thereof[,] and ... mixtures thereof." '659 patent, col. 28, ll. 31-39. Claim 6 is also similarly directed to the three deposited sequences and subsequences and mutated variations thereof. *Id.* at ll. 47-56. The specification defines a subsequence non-specifically as a nucleotide sequence "greater than about 12 nucleotides." '659 patent, col. 3, ll. 29-30. As the deposited sequences are about 850, 850, and 1300 nucleotides long, *id.* at col. 13, ll. 47-49, there are at least hundreds of subsequences of the deposited sequences, an unknown number of which might also meet the claimed hybridization ratio. Moreover, Enzo's expert, Dr. Wetmur, stated that "astronomical" numbers of mutated variations of the deposited sequences also fall within the scope of those claims, and that such broad claim scope is necessary to adequately protect Enzo's invention from copyists who could otherwise make a minor change to the sequence and thereby avoid infringement while still exploiting the benefits of Enzo's invention. The defendants assert that such breadth is fatal to the adequacy of the written description. On the other hand, because the deposited sequences are described by virtue of a reference to their having been deposited, it may well be that various subsequences, mutations, and mixtures of those sequences are also described to one of skill in the art. We regard that question as an issue of fact that is best resolved on remand. [FN2] Because the district court's grant of summary judgment was based on its conclusion that Enzo's deposits could not satisfy the written description requirement as a matter of law, we reverse the district court's grant of summary judgment that claims 4 and 6 are invalid for failure to meet the written description requirement. On remand, the court should determine whether a person of skill in the art would glean from the written description, including information obtainable from the deposits of the claimed sequences, subsequences, mutated variants, and mixtures sufficient to demonstrate possession of the generic scope of the claims.

> FN2. We do not address the issue whether the breadth of the claim may implicate other validity issues, such as enablement. Only written description is before us.

[10] We next address the question whether the compositions of the broader genus claims 1-3 and 5

are sufficiently ***967** described to meet the requirements of § 112, ¶ 1, on the basis of Enzo's deposits of three sequences. If those sequences are representative of the scope of the genus claims, *i.e.,* if they indicate that the patentee has invented species sufficient to constitute the genera, they may be representative of the scope of those claims. *See In re Smythe,* 480 F.2d 1376, 1383, 178 USPQ 279, 284-85 (CCPA 1973) (discussing circumstances in which a species may be representative of and therefore descriptive of genus claims). Because the district court concluded that the deposited sequences were not themselves described, it did not determine whether that description was representative of the genera in those claims. Such determination should be made on remand.

When we addressed a similar issue in *Eli Lilly,* we determined that a disclosure of the sequence of rat cDNA was not descriptive of the broader invention consisting of mammalian and vertebrate cDNA, although it was a species falling within the scope of those claims. *Eli Lilly,* 119 F.3d at 1567-68, 43 USPQ2d at 1405. In *Eli Lilly,* the specification and generic claims to all cDNAs encoding for vertebrate or mammalian insulin did not describe the claimed genus because they did not set forth any common features possessed by members of the genus that distinguished them from others. *Id.* at 1568, 43 USPQ2d at 1405. Nor did the specification describe a sufficient number of species within the very broad genus to indicate that the inventors had made a generic invention, *i.e.,* that they had possession of the breadth of the genus, as opposed to merely one or two such species. *Id.* The PTO has included a hypothetical example based on the facts of *Eli Lilly* in its Synopsis of Application of Written Description Guidelines in which the description requirement is not met. *See Application of Guidelines,* Example 17, at 61-64. The PTO has also provided a contrasting example of genus claims to nucleic acids based on their hybridization properties, and has determined that such claims may be adequately described if they hybridize under highly stringent conditions to known sequences because such conditions dictate that all species within the genus will be structurally similar. *See id.,* Example 9, at 35-37. Whether the disclosure provided by the three deposits in this case, coupled with the skill of the art, describes the genera of claims 1- 3 and 5 is a fact question the district court did not address. On remand, the district court should determine, consistently with the precedent of this court and the PTO's Guidelines, whether one skilled in the art would consider the subject matter of claims 1-3 and 5 to be adequately described, recognizing the significance of the deposits and the scope of the claims.

[11] Enzo argues that all of the claims are adequately described on another basis, *viz.,* by means of the disclosed correlation of the function of hybridization with the bacterial DNA. In its petition for rehearing, Enzo states as attorney argument that "[t]he description and claiming of biological materials by their affinity to other materials that are clearly identified in the specification and claims (the particular deposited strains of *N. gonorrhoeae* and *N. meningitidis*) inherently specifies structure, and is routine in this field." Claim 1 sets forth the deposit numbers of six strains of *N. gonorrhoeae* to which the claimed nucleotide sequences preferentially hybridize, as well as the deposit numbers of six strains of *N. meningitidis* that are thereby distinguished. Again, as with the claimed nucleotide sequences, the sequences of the genomic DNA of those bacteria are not disclosed, perhaps because such sequencing would have been unduly burdensome at the time of Enzo's invention. ***968** 659 patent, col. 3, ll. 40-46 (noting that it would take 3,000 scientists one month to sequence the genome of one strain of *N. gonorrhoeae* and one strain of *N. meningitidis*). However, as those bacteria were deposited, their bacterial genome is accessible and, under our holding today, they are adequately described in the specification by their accession numbers. Because the claimed nucleotide sequences preferentially bind to the genomic DNA of the deposited strains of *N. gonorrhoeae* and have a complementary structural relationship with that DNA, those sequences, under the PTO Guidelines, may also be adequately described. Although the patent specification lacks description of the location along the bacterial DNA to which the claimed sequences bind, Enzo has at least raised a genuine issue of material fact as to whether a reasonable fact-finder could conclude that the claimed sequences are described by their ability to hybridize to structures that, while not explicitly sequenced, are accessible to the public. Such hybridization to disclosed organisms may meet the PTO's Guidelines stating that functional claiming is permissible when the claimed material hybridizes to a disclosed substrate. That is a fact question. We therefore conclude that the district court erred in granting summary judgment that the claims are invalid for failure to meet the written description requirement. On remand, the court should consider whether one of skill in the art would find the generically claimed sequences described on the basis of Enzo's disclosure of the hybridization function and an accessible structure,

consistent with the PTO Guidelines. If so, the written description requirement would be met.

[12][13][14] We next address Enzo's additional argument that the written description requirement for the generic claims is necessarily met as a matter of law because the claim language appears *in ipsis verbis* in the specification. We do not agree. Even if a claim is supported by the specification, the language of the specification, to the extent possible, must describe the claimed invention so that one skilled in the art can recognize what is claimed. The appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. One may consider examples from the chemical arts. A description of an anti-inflammatory steroid, *i.e.,* a steroid (a generic structural term) described even in terms of its function of lessening inflammation of tissues fails to distinguish any steroid from others having the same activity or function. Similarly, the expression an antibiotic penicillin fails to distinguish a particular penicillin molecule from others possessing the same activity. A description of what a material does, rather than of what it is, usually does not suffice. *Eli Lilly,* 119 F.3d at 1568, 43 USPQ2d at 1406. The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. *Id.*

[15][16] In *Eli Lilly,* we were faced with a set of facts in which the words of the claim alone did not convey an adequate description of the invention. *Id.* at 1567, 119 F.3d 1559, 43 USPQ2d at 1405. In such a situation, regardless whether the claim appears in the original specification and is thus supported by the specification as of the filing date, § 112, ¶ 1 is not necessarily met. *See Guidelines* at 1100 (noting *Eli Lilly's* clarification of the "original claim" doctrine in situations in which the name of the claimed material does not convey sufficient identifying information). If a purported description of an invention does not meet the requirements of the statute, the fact that it appears as an original claim or in the specification does ***969** not save it. A claim does not become more descriptive by its repetition, or its longevity.

[17] Inasmuch as 112, 1 requires such description, we are not persuaded by Enzo's argument that, because the specification indicated that Enzo possessed the claimed invention by reducing three sequences within the scope of the claims to practice, Enzo necessarily described the invention. It is true that in *Vas-Cath,* we stated: "The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." *Vas-Cath,* 935 F.2d at 1563-64, 19 USPQ2d at 1117. That portion of the opinion in *Vas-Cath,* however, merely states a *purpose* of the written description requirement, *viz.,* to ensure that the applicant had possession of the invention as of the desired filing date. It does not state that possession alone is always sufficient to meet that requirement. Furthermore, in *Lockwood v. American Airlines, Inc.,* we rejected Lockwood's argument that "all that is necessary to satisfy the description requirement is to show that one is 'in possession' of the invention." 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed.Cir.1997). Rather, we clarified that the written description requirement is satisfied by the patentee's disclosure of "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention." *Id.*

[18][19][20] The articulation of the written description requirement in terms of "possession" is especially meaningful when a patentee is claiming entitlement to an earlier filing date under 35 U.S.C. § § 119 or 120, in interferences in which the issue is whether a count is supported by the specification of one or more of the parties, and in *ex parte* applications in which a claim at issue was filed subsequent to the application. *See Vas- Cath,* 935 F.2d at 1560, 19 USPQ2d at 1114 (describing situations in which the written description requirement may arise); *Ralston Purina Co. v. Far-Mar- Co, Inc.,* 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed.Cir.1985) (noting, in the context of claiming entitlement to the priority date of an earlier application, that the written description requirement is met if "the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter"). Application of the written description requirement, however, is not subsumed by the "possession" inquiry. A showing of "possession" is ancillary to the *statutory* mandate that "[t]he specification shall contain a written description of the invention," and that requirement is not met if, despite a showing of possession, the specification does not adequately describe the claimed invention. After all, as indicated above, one can show possession of an invention by means of an affidavit or declaration during prosecution, as one does in an interference or when one files an affidavit under 37 C.F.R. § 1.131 to antedate a reference. However,

such a showing of possession alone does not cure the lack of a written description in the specification, as required by statute.

[21][22] Similarly, we conclude that proof of a reduction to practice, absent an adequate description in the specification of what is reduced to practice, does not serve to describe or identify the invention for purposes of § 112, ¶ 1. As with "possession," proof of a reduction to practice may show priority of invention or allow one to antedate a reference, but it does not by itself provide a written description *in the patent specification.* We are thus not persuaded ***970** by Enzo's argument, relying on the PTO's Guidelines, that its disclosure of an actual reduction to practice is an important "safe haven" by which it has demonstrated compliance with the description requirement. The Guidelines state:

> Actual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps, in such a way as to distinguish the composition with particularity from all others.

Guidelines, 66 Fed. Reg. at 1101. For biological inventions, for which providing a description in written form is not practicable, one may nevertheless comply with the written description requirement by publicly depositing the biological material, as we have held today. That compliance is grounded on the fact of the deposit and the accession number in the specification, not because a reduction to practice has occurred. Such description is the *quid pro quo* of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time.

CONCLUSION

For the foregoing reasons, we conclude that the district court erred in granting summary judgment that the claims of the 659 patent are invalid for failure to meet the written description requirement of 112, 1. While the district judge clearly understood and correctly applied this courts existing precedent, we nevertheless reverse because this case has taken us into new territory and we have held, as a matter of first impression, that reference in a patent specification to a deposit of genetic material may suffice to describe that material. We therefore remand for further resolution consistent with this opinion.

REVERSED and REMANDED

ORDER
July 15, 2002.

A petition for rehearing was filed by the plaintiff-appellant, and a response thereto was invited by the court and filed by the defendants-appellees. The United States Patent and Trademark Office and Fish & Richardson P.C. filed briefs as amici curiae. This matter was referred first to the merits panel that heard this appeal, which vacated its earlier decision and prepared a revised decision for issuance. Thereafter, at the request of a non-panel judge, an en banc poll was conducted concerning whether the appeal ought to be heard en banc. The poll failed. *Circuit Judges* RADER, GAJARSA, and LINN would have heard the appeal en banc.

Upon consideration thereof,

IT IS ORDERED THAT:

The petition for rehearing is granted as set forth in the panel opinion issued concurrently with this order.

LOURIE, Circuit Judge, with whom PAULINE NEWMAN, Circuit Judge, joins, filed an opinion concurring in the court's decision not to hear the case en banc.

PAULINE NEWMAN, Circuit Judge, filed an opinion concurring in that decision.

DYK, Circuit Judge, filed an opinion concurring in that decision.

RADER, Circuit Judge, with whom GAJARSA and LINN, Circuit Judges, join, filed an opinion dissenting from that decision.

***971** LINN, Circuit Judge, with whom RADER and GAJARSA, Circuit Judges, join, filed an opinion dissenting from that decision.

ON DENIAL OF PETITION FOR REHEARING EN BANC

LOURIE, Circuit Judge, with whom PAULINE NEWMAN, Circuit Judge, joins, concurring in the court's decision not to hear the case en banc.

I agree that the court correctly declined to hear this case *en banc.*

First, it is important to note that the earlier panel majority, in response to the petition for rehearing, has reversed its earlier decision. Taking the case *en banc* would therefore delay and hence frustrate the remand of the case solely for the purpose of revising written description law. That law is sound and does not need revision, at least as proposed by the dissents.

The dissenters believe that the written description requirement is simply a requirement for enablement. With all due respect, that is incorrect. The *complete* statutory provision is as follows:

> *The specification shall contain a written description of the invention,* and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 U.S.C. § 112, ¶ 1 (1994) (emphasis added). I read the statute so as to give effect to its language. The statute states that the invention must be described. That is basic patent law, the *quid pro quo* for the grant of a patent. Judge Rader notes that historically the written description requirement served a purpose when claims were not required. While that may be correct, when the statute began requiring claims, it was not amended to delete the requirement; note the comma between the description requirement and the enablement provision, and the "and" that follows the comma. Judge Rich, whom Judge Rader cites, was in fact one of the earliest interpreters of the statute as having separate enablement and written description requirements. *In re Ruschig,* 54 C.C.P.A. 1551, 379 F.2d 990, 995-996, 154 USPQ 118, 123 (C.C.P.A.1967); *Vas-Cath Inc. v. Mahurkar,* 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1117 (Fed.Cir.1991). The basic requirement to describe one's invention was recently emphasized as an independent patentability requirement by the Supreme Court in *Festo:*

> In addition, the patent application must *describe,* enable, and set forth the best mode of carrying out the invention. § 112 (1994 ed.). These latter requirements must be satisfied before issuance of the patent, for exclusive patent rights are given in exchange for *disclosing* the invention to the public. *See Bonito Boats,* 489 U.S. at 150-151, 109 S.Ct. 971. *What is claimed by the patent application must be the same as what is disclosed in the specification; otherwise the patent should not issue.*

Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., 535 U.S. 722, 122 S.Ct. 1831, 1840, 152 L.Ed.2d 944 (2002) (emphases added).

It is said that applying the written description requirement outside of the priority context was novel until several years ago. Maybe so, maybe not; certainly such a holding was not precluded by statute or precedent. New interpretations of old statutes in light of new fact situations occur all the time. I believe these issues have arisen in recent years for the same reason that more doctrine of equivalents ***972** issues are in the courts, *viz.,* because perceptions that patents are stronger tempt patent owners to try to assert their patents beyond the original intentions of the inventors and their attorney. That is why the issues are being raised and that is why we have to decide them. Claims are now being asserted to cover what was not reasonably described in the patent.

Moreover, the dissenters would limit the requirement, to the extent that they credit the written description portion of the statute as being a separate requirement at all, to priority issues. The statute does not say "a written description of the invention for purposes of policing priority." While it has arisen primarily in cases involving priority issues, Congress has not so limited the statute, and we have failed to so limit it as well. As for the lack of earlier cases on this issue, it regularly happens in adjudication that issues do not arise until counsel raise them, and, when that occurs, courts are then required to decide them. Even now, a written description issue should not arise unless a patentee seeks to have his claims interpreted to include subject matter that he has not adequately disclosed in his patent. Although it is true that the written description requirement has been applied rigorously in some recent cases, I do not believe that any of those cases were decided wrongly. The losing patents (or applications) involved did not adequately disclose what was claimed: a particular ratio of variables, *Purdue Pharma L.P. v. Faulding Inc.,* 230 F.3d 1320, 1327, 56 USPQ2d 1481, 1487 (Fed.Cir.2000); a sofa with controls other than on the console, *Gentry Gallery, Inc. v. Berkline Corp.,* 134 F.3d 1473, 1480, 45 USPQ2d 1498, 1503- 1504 (Fed.Cir.1998); a cup other than a conical cup,

Tronzo v. Biomet, Inc., 156 F.3d 1154, 1159-60, 47 USPQ2d 1829, 1833-34 (Fed.Cir.1998); human insulin cDNA, *Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1567, 43 USPQ2d 1398, 1405 (Fed.Cir.1997); or beta-interferon, *Fiers v. Revel*, 984 F.2d 1164, 1171, 25 USPQ2d 1601, 1606 (Fed.Cir.1993). Interpretation of written description as this court has done furthers the goal of the law to have claims commensurate in scope with what has been disclosed to the public.

I believe that the dissenters miss the point in seeing this case as involving an original claim or *in ipsis verbis* issue. There is no question that an original claim is part of the specification. That was the question answered in the affirmative by *In re Gardner*, 480 F.2d 879, 178 USPQ 149 (C.C.P.A.1973), in which the CCPA found compliance with the written description requirement over the objection of the PTO Commissioner, who argued that an original claim should not be considered part of the written description unless the specification was amended to contain the subject matter of the original claim. However, the question here is whether the disclosure, as an original claim, or in the specification, [FN*] adequately describes the invention. It is incorrect that the mere appearance of vague claim language in an original claim or as part of the specification necessarily satisfies the written description requirement or shows possession of a generic invention.

> FN* Enzo's claim 1 is actually not an original claim. It was amended to include language appearing in the original specification and it thus appears *in ipsis verbis* in the specification.

Not only are we not entitled to ignore the statutory written description requirement, but our court has not. Earlier cases also upheld a separate written description requirement, and the fact that they may have pertained to priority disputes does not vitiate their basic requirement to disclose one's invention. Section 112, paragraph ***973** 1, does not limit itself to priority disputes. I thus believe it is incorrect, as Judge Rader states, that our cases have limited the written description/new matter doctrine to priority protection. Opinions explain the decisions on the issues that come before them on the facts presented; those cases have not expressly limited the written description requirement to priority issues, and in fact they emphasize that the requirement arises in a "variety of situations." *In re Wright*, 866 F.2d 422, 424, 9 USPQ2d 1649, 1651 (Fed.Cir.1989). Any language seemingly appearing to limit the language to priority issues does so because it addresses a priority issue that was before it. Other broad language is not binding holding on different facts and raising different issues. Courts do not, or should not, purport to write treatises on the law, outlining all aspects of a statute that comes before them. They decide issues raised in light of the decision being reviewed.

Moreover, even if written description is related to and overlaps with "new matter," so what? One can fail to meet the requirements of the statute in more than one manner, and in any event the case cited as equating those two requirements in fact distinguishes § § 112 and 132 as concerning: (1) claims not supported by the disclosure; and (2) the prohibition of new matter to the disclosure, respectively. *In re Rasmussen*, 650 F.2d 1212, 1214-15, 211 USPQ 323, 326 (C.C.P.A.1981). *Rasmussen* states that "[t]he proper basis for rejection of a claim amended to recite elements thought to be without support in the original disclosure, therefore, is § 112, first paragraph, not § 132." *Id.*

In addition, we do not "elevate 'possession' to the posture of a statutory test of patentability." Rather, the opinion refines the "possession" test for circumstances such as these in which the inventors showed possession of a species of the invention by reference to a deposit, but may not have described what else within the scope of the claims they had possession of. While "possession" is a relevant factor in determining whether an invention is described, it is only a criterion for satisfying the statutory written description requirement. Showing possession is not necessarily equivalent to providing a written description.

Judge Rader's dissenting opinion cites authors who disapprove of our decisions. While views of knowledgeable and objective commentators are surely of interest to this court, we should not interpret the law based on taking polls of discontented writers. Our commission is to apply the law to the facts and attempt to explain the reasons for our decisions. Critical articles may be written by those who have lost a case, or those who are skilled in a particular technology or not, or those who have little practical experience or who are seasoned experts. While Judge Rader cites articles critical of *Lilly*, others are favorable. Not surprisingly, an author from Eli Lilly took a positive view of the case. *See* Mark J.

Stewart (patent associate at Eli Lilly), Note, *The Written Description Requirement of 35 U.S.C. § 112(1): The Standard After Regents of the University of California v. Eli Lilly & Co.*, 32 Ind. L.Rev. 537, 563 (1999) ("[T]he holding in *Lilly* actually avoided a disaster that would have crippled the biotechnology industry. The enormous amount of time and money companies spend to study DNA and protein variants, to clone homologous genes and protein family members, and to mine databases would no longer be justified had the court found the written description in '525 adequate.").

Other authors support a robust written description requirement and point out the benefits of such a requirement to the public. ***974** *See, e.g.*, Scott A. Chambers, *"Written Description" and Patent Examination Under the U.S. Patent and Trademark Office Guidelines,* IP Litigator, Sept.-Oct.2000, at 9, 10 ("Thus, the Federal Circuit's present interpretation of the written description requirement maintains the vitality of the U.S. patent system and provides disclosures that others can build on. By suggesting that disclosure of the structure or actual sequence of complex chemical entities may sometimes be required, the Federal Circuit may have advanced the goal of the patent system to actually put the claimed invention into the hands of the public."); Margaret Sampson, *The Evolution of the Enablement and Written Description Requirements under 35 U.S.C. § 112 in the Area of Biotechnology,* 15 Berkeley Tech. L.J. 1233, 1260-61 (2000) ("Without a heightened written description requirement, inventors could receive patent rights to sequences of which they have no knowledge, in organisms with which they have never worked.... Therefore, the Federal Circuit's approach to the written description requirement in the area of biotechnology has prevented nucleotide sequence claims from becoming a Pandora's box that the patent law is unable to control."). In any event, we decide cases as they come to us, based on the arguments raised, the decisions below, the law, the facts, and our best efforts, not based on occasional journal articles.

Since some of the cases implicated by this issue are in the biotech field, I should point out that, among the problems in comprehension of the issues in a biotech context is that a functional description of DNA does not indicate which DNA has been invented. And simply acknowledging the presence of a DNA that serves a particular function, whose existence has been postulated since, perhaps, Mendel, plus a general process for finding it, is not a description of the DNA. It is a research plan at best, and does not show "possession" of any invention.

Still, in terms of the more practical aspects of complying with the statute, meeting the description requirement is the first task in drafting a patent application. Enabling one of skill in the art to make and use the invention is a separate requirement. To interpret the written description requirement only as an enablement provision is to let the tail wag the dog. Perhaps there is little difference in electrical and mechanical inventions between describing an invention and enabling one to make and use it, but that is not true of chemical and chemical-like inventions.

Enzo's patent claimed a genus of chemical-like materials (a sequence of nucleic acids is of a chemical nature--note the claims begin with "a composition"). Although one may envision a general concept, what one usually does first in making or isolating a chemical or chemical-related invention is to obtain a specific material or materials. One then broadens the concept to extend it as far as one envisions that other materials will have the same utility and can be similarly made. That broadened concept becomes the genus in a patent application that is both the broadest statement constituting a written description and usually claim 1. One then elaborates to fill in the genus with representative examples of compounds or substances that fall within the genus. That is part of the written description needed to support the generic claim. *Then,* one tells how to make the materials, and then how to use them. That is enablement, separate in concept from describing *what* the invention is. The idea that there is no requirement in the statute to describe one's new invention (aside from the fact that the language of the statute contains one) separate from the requirement to enable one to make and use it is not correct. Disclosure ***975** is the first role of a patent. One must first state what one's invention is. That is quite different from telling how to make and use it.

Some commentators have had difficulty in understanding how one may have enabled an invention, but not described it. They believe they must coincide. As an example of how the written description and enablement provisions differ in chemistry, however, one may readily have enabled the making of an invention, but still not have described it. For example, a propyl or butyl compound may be made by a process analogous to a disclosed methyl compound, but, in the absence of a statement that the propyl and butyl compounds are part of the invention, they have not been described

and they are not entitled to a patent (I make no implication here about coverage under the doctrine of equivalents). *See In re DiLeone*, 58 C.C.P.A. 925, 436 F.2d 1404, 1405 n. 1, 168 USPQ 592, 593 n. 1 (1971) ("[C]onsider the case where the specification discusses only compound A and contains no broadening language of any kind. This might very well enable one skilled in the art to make and use compounds B and C; yet the class consisting of A, B and C has not been described."). This is surely part of the recent history of some biotechnology patents.

In sum, we have evolved a consistent body of law over a number of years, based on the statute and basic principles of patent law. I see no reason to hear this case *en banc* and rewrite the statute.

PAULINE NEWMAN, Circuit Judge, concurring in the denial of rehearing en banc.

I join Judge Lourie's statement, and write separately to emphasize my concern with the position of the dissent concerning the law of written description. The description of the invention has always been the foundation of the patent specification. It sets forth what has been invented, and sets boundaries of what can be claimed. The theory of the dissent that a description of the invention is not needed in order to support the claims, but serves only to antedate prior art or establish priority in an interference, is a dramatic innovation in the theory and practice of patents. It has never been the sole purpose of the description requirement, and negates not only the logic but also the history of patent practice. The dissent's citation of cases in which the description of the invention has been relied on to antedate references and in interference contests reinforces, not reduces, the role of the description of the invention in establishing what has been invented.

The dissent argues that the subject matter that is intended to be patented need not be described, as long as it is enabled. Undoubtedly, in many patents these requirements are met by the same information content. And the special case of the biological deposit is a method of complying with the statutory requirements, as the panel now confirms; this expedient implements the statute for this special subject matter, but does not change it. It is not the law that the description of the invention serves only to establish priority, to be invoked only when priority is at issue. The invention that is covered by the claims must be described as well as enabled, as the statute has always required.

DYK, Circuit Judge, concurring in the court's decision not to hear the case en banc.

The opinions of Judges Newman, Lourie, Rader, and Linn concerning the denial of *en banc* rehearing raise important and interesting questions, including questions concerning the correctness of our earlier ***976** decision in *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed.Cir.1997), *cert. denied*, 523 U.S. 1089, 118 S.Ct. 1548, 140 L.Ed.2d 695 (1998), that may someday warrant the court's *en banc* attention. Given the panel's decision on rehearing, remanding for further consideration by the district court, this is not the appropriate occasion for *en banc* review. The court will also benefit from further percolation of these issues before they are addressed by the full court.

RADER, Circuit Judge, with whom GAJARSA and LINN, Circuit Judges, join, dissenting from the court's decision not to hear the case en banc.

The tortuous path of this case shows the perils of ignoring the statute and over thirty years of consistent written description case law [FN1]. The first version of this opinion, *Enzo Biochem, Inc. v. **Gen-Probe**, Inc.*, 285 F.3d 1013, 62 USPQ2d 1289 (Fed.Cir.2002), purported to invalidate a patent because the inventor had not shown "possession of the invention" for written description. *See, Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1564, 19 USPQ2d 1111, 1117 (Fed.Cir.1991). As this court now acknowledges, an inventor can hardly show possession of an invention better than by depositing the invention in an internationally recognized repository. This court corrects part of the mistake of *Enzo I.* Yet the court still remands to the district court to reexamine the written description requirement. Because the written description requirement as created and applied for thirty years does not apply to this case, I would grant *en banc* review and correct the rest of this court's misapplication of the description requirement.

FN1. An appendix at the close of this opinion will briefly explicate all written description cases from its creation in 1967 in the Court of Customs and Patent Appeals to

the present. This appendix shows that only two cases, this ENZO case and the 1997 LILLY case have purported to apply the doctrine outside its purpose and function.

Statute

Because the greater mistake in this case is misapplication of this court's written description case law, this opinion devotes only a few paragraphs to the statutory interpretation question. The United States' brief as *amicus curiae* in support of rehearing *en banc* states concisely this *Enzo* opinion's disregard for the statute:

> A straightforward reading of the text of section 112 suggests that the test for an adequate written description is whether it provides enough written information for others to make and use the invention. The statute provides that the "specification shall contain a written description of the invention ... in such full, clear, concise, and exact terms as to enable any person skilled in the art ... to make and use the same." 35 U.S.C. § 112 ¶ 1. Thus, an adequate written description assures that others can "make and use" the invention. [FN2]

> FN2. This court rejected the "straightforward reading" of the statute in *Vas-Cath* because the written description (WD) doctrine was a priority control, not the general disclosure doctrine of enablement. *See, Vas- Cath,* 935 F.2d 1555. Within the proper purpose of WD, *Vas-Cath* makes sense. When applied outside the priority context as a general disclosure doctrine, however, WD cannot depart from the enablement test without replacing it. Thus, the United States advocates application of the statutory standard of enablement.

If it is possible to characterize disregard of statutory text as a secondary mistake, this case fits that classification. The more important problem is disregard for the case law that originated the written description requirement and applied it for over thirty years.

977 *Origin and History of the Written Description Requirement

The words "written description" first appeared in the Patent Act of 1793. At that time, of course, patents did not require claims but only a written description sufficient "to distinguish [the invention] from all other things before known or used." In *Evans v. Eaton,* 20 U.S. (7 Wheat.) 356, 5 L.Ed. 472 (1822), the Supreme Court construed the description language to require applicants to enable their inventions and to provide the notice function of claims:

> [After enablement,] [t]he other object of the specification is to put the public in possession of what the party claims as his own invention, so as to ascertain if he claims any thing that is in common use, or is already known ...

Id. at 433. In later enactments, this notice function was assigned to claims, leaving enablement as the only purpose of the "written description" language. As noted in the United States' brief, the modern descendant of the 1793 phrase still requires only a written description "in such ... terms as to enable [the invention]." 35 U.S.C. § 112. In *J.E.M. AG Supply,* the Supreme Court acknowledged only enablement as the disclosure *quid pro quo* of the Patent Act: "In addition [to novelty, utility, and nonobviousness], to obtain a utility patent, a breeder must describe the plant with sufficient specificity to enable others to 'make and use' the invention after the patent term expires." *J.E.M. AG Supply, Inc. v. Pioneer Hi-Bred Int'l, Inc.,* 534 U.S. 124, 122 S.Ct. 593, 604, 151 L.Ed.2d 508 (2001). Reading the statute, the Supreme Court correctly found no general disclosure requirement in title 35 other than enablement. [FN3]

> FN3. In *Festo,* the Supreme Court mentions a description requirement separate from enablement. *Festo Corp. v. Shoketsu Kogyo Kabushiki Co.,* 535 U.S. 722, 122 S.Ct. 1831, 1840, 152 L.Ed.2d 944 (2002). This listing of doctrines, however, did not endorse any departure from this court's case law for more than thirty years.

Before 1967, this court's predecessor, the United States Court of Customs and Patent Appeals also did not differentiate written description from enablement. In 1966, that predecessor court wrote in detail about section 112, paragraph 1, and found only two requirements--enablement (the A requirement under Judge Rich's terminology) and best mode (the B requirement). *In re Gay,* 50 C.C.P.A. 725, 309 F.2d 769, 772, 135 USPQ 311, 315 (1962).

In 1967, the Court of Customs and Patent Appeals first separated a new written description (WD)

requirement from the enablement requirement of § 112. The reason for this new judge-made doctrine needs some explanation. Every patent system must have some provision to prevent applicants from using the amendment process to update their disclosures (claims or specifications) during their pendency before the patent office. Otherwise applicants could add new matter to their disclosures and date them back to their original filing date, thus defeating an accurate accounting of the priority of invention. Priority always a vital issue in patent prosecution procedures--often determines entitlement to an invention. Before 1967, the United States Patent Office and the Court of Customs and Patent Appeals used a "new matter" rejection to ensure that applicants did not update their disclosures after the original filing date of the application. This "new matter" rejection had a statutory basis: "No amendment shall introduce new matter into the disclosure of the invention." 35 U.S.C. § 132.

In 1967, in *In re Ruschig*, 54 C.C.P.A. 1551, 379 F.2d 990, 154 USPQ 118 (1967), this court's predecessor created for the ***978** first time a new WD doctrine to enforce priority. In the context of a new claim added "[a]bout a year after the present application was filed," the *Ruschig* court sought to determine "whether [the new] claim 13 is supported by the disclosure of appellants' application." *Id.* at 991. Rather than use § 132, however, *Ruschig* assigned the role of policing priority to § 112. As a technical matter, the Court of Customs and Patent Appeals distinguished between adding new matter to the specification and adding new matter to the claims. Under PTO practice, new matter in the claims would draw a § 132 *rejection* of the claims; new matter in the specification would draw a § 132 *objection* to the addition. The *Ruschig* court, for the first time, decided to treat the objection alone as a § 132 matter. To deal with new matter in the claims, the court calved a new WD doctrine out of the § 112 enablement requirement [FN4]. As long as the new WD doctrine applied according to its original purpose as an identical twin of the § 132 new matter doctrine, these technical distinctions were of little practical consequence.

> FN4. As a matter of integrity to the statute, the *Ruschig* distinction has a major problem, namely the language of § 132 embraces both new matter rejections of amended claims and new matter objections to amended specifications. Both claims and the rest of the specification are part of the patent "disclosure" within the terms of § 132. *See, e.g., In re Frey*, 35 C.C.P.A. 970, 166 F.2d 572, 575, 77 USPQ 116, 119 (1948) ("Certainly the [claim] is a disclosure of itself."). Moreover implicit in the judicial creation of a new WD requirement is the incorrect assumption that the Patent Act had no remedy for new matter in claims before 1967. In fact, § 132 embraces both new matter rejections and objections.

In any event, the WD doctrine, at its inception had a very clear function preventing new matter from creeping into claim amendments. Judge Rich, the author of *Ruschig*, often reiterated the purpose of WD. For instance in the case of *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), the Court of Customs and Patent Appeals confronted a priority issue:

> The dispositive issue under this heading is whether appellants' parent and Swiss applications comply with 35 U.S.C. § 112, first paragraph, including the description requirement, as to the subject matter of these claims. If they do, these claims are entitled to the filing dates of the *parent* application.... [A] right of foreign *priority* in appellants' Swiss application will antedate Pfluger 1966 and remove it as prior art against the claims.

Id. at 261 (emphasis added). In resolving this question, Judge Rich stated again the purpose of WD: "The function of the description requirement is to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter *later* claimed by him." *Id.* at 262 (emphasis added). In sum, WD was a new matter doctrine, a priority policeman.

Returning to the history of WD, after 1967, the PTO continued to use new matter rejections under § 132, but also embraced the coterminous written description analysis. Thus, for many years, the PTO rejected priority errors in claims under both § 132 and § 112.

In 1981, the Court of Customs and Patent Appeals noted that the two rejections were interchangeable: "This court, ha[s] said that a rejection of an amended claim under § 132 is *equivalent to* a rejection under § 112, first paragraph." *In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 325 (CCPA, 1981) (emphasis added). To avoid confusion between new matter rejections and objections, the court chose to eliminate the § 132/§ 112 rejections and to use § 112 for new matter rejections (claims): "The proper

basis for rejection of a claim amended to recite elements ***979** thought to be without support in the original disclosure, therefore, is § 112, first paragraph, not § 132." *Id.* The purpose of the doctrine did not change. As the sentence above states explicitly, the § 112 doctrine, like its corollary § 132, policed priority, nothing more. At no time did either the CCPA or the Federal Circuit purport to apply the equivalent new matter/written description rejections to original claims or other claims without priority problems. *See, e.g., In re Koller,* 613 F.2d 819, 823, 204 USPQ 702, 706 (CCPA 1980) ("[O]riginal claims constitute their own description."); *In re Gardner,* 475 F.2d 1389, 1391, 177 USPQ 396, 397 (CCPA 1973) ("Claim 2, which apparently was an original claim, in itself constituted a description in the original disclosure.... Nothing more is necessary for compliance with the description requirement...."). WD, the equivalent of the statutory new matter doctrine, simply has no application to claims without priority problems.

The Federal Circuit continued to follow this binding precedent. *See, e.g., Vas-Cath,* 935 F.2d at 1560 ("The question raised by these situations is most often phrased as whether the application provides 'adequate support' for the claim(s) at issue; it has also been analyzed in terms of 'new matter' under 35 U.S.C. § 132."); *In re Wright,* 866 F.2d 422, 424, 9 USPQ2d 1649, 1651 (Fed.Cir.1989) ("When the scope of a claim has been changed by amendment in such a way as to justify an assertion that it is directed to a *different invention* than was the original claim, it is proper to inquire whether the newly claimed subject matter was *described* in the patent application when filed as the invention of the applicant. That is the essence of the so-called 'description requirement' of § 112, first paragraph.") (emphases added); [FN5] *In re Kaslow,* 707 F.2d 1366, 217 USPQ 1089 (Fed.Cir.1983). In fact, this Circuit's test for written description required assessment of the specification to check "later claimed subject matter." *Id.* at 1375 ("The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the *later* claimed subject matter, *rather than the presence or absence of literal support in the specification for the claim language.*") (emphasis added). In fact, this standard emphasizes that WD does not examine the specification for "literal support" of the claim language unless priority is in question. In any event, this Circuit did not apply WD to claims without priority problems because the doctrine had no purpose beyond policing priority. [FN6]

FN5. In *Wright,* Judge Rich mentions that WD arises in "a variety of situations." *Id.* Of course, this observation is an accurate description of the priority issue. Priority arises in the context of a § 102(b) rejection, *see, e.g., In re Ruschig,* 379 F.2d at 991, a § 119 issue, *see, e.g., In re Wertheim,* 541 F.2d at 261, a § 120 issue, *see, e.g., Kennecott Corp. v. Kyocera Int'l., Inc.,* 835 F.2d 1419, 1421 (Fed.Cir.1987) ("The incorporation of the requirements of section 112 into section 120 ensures that the inventor had possession of the later- claimed invention on the filing date of the earlier application."), and a § 102(g) interference, *see, e.g., Fiers v. Revel,* 984 F.2d at 1169, to mention just a few of the variety of situations in which priority arises. This statement hardly justifies applying WD outside its purpose as a test for sufficiency of disclosure.

FN6. Again, the appendix at the close of this opinion shows that the Federal Circuit uniformly applies WD to police priority. Only the LILLY and this ENZO opinion purport to apply it as a general disclosure requirement in place of enablement.

The deviation from thirty years of precedent

In 1997, for the first time, this court purported to apply WD as a general disclosure ***980** doctrine in place of enablement, rather than as a priority doctrine. *Regents of the Univ. of Cal. v. Eli Lilly and Co.,* 119 F.3d 1559, 43 USPQ2d 1398 (Fed.Cir.1997). In *Lilly,* this court found that the '525 patent specification does not provide a WD of human insulin cDNA despite the disclosure of a general method of producing human insulin cDNA and a description of the human insulin A and B chain amino acid sequences that cDNA encodes. 119 F.3d at 1567. In the words of the court, "a description that does not render a claimed invention obvious does not sufficiently describe that invention for purposes of § 112, ¶ 1." *Id.* At another point, the court stated: "An adequate written description of a DNA ... 'requires a precise definition, such as by structure, formula, chemical name, or physical properties ....' " *Id.* at 1566 (quoting *Fiers v. Revel,* 984 F.2d 1164,

1171, 25 USPQ2d 1601, 1606 (Fed.Cir.1993)). In sum, the *Lilly* opinion does not test a later claim amendment against the specification for priority, but asserts a new free-standing disclosure requirement in place of the statutory standard of enablement. Based on the absence of a nucleotide-by-nucleotide recitation in the specification of the human insulin cDNA, the court determined that the applicant had not adequately described the invention. For the first time, this court purported to apply WD without any priority question. *But see, Kaslow,* 707 F.2d at 1375 ("rather than the presence or absence of literal support in the specification for the claim language."). Even accepting that WD can be isolated as a separate requirement from enablement in § 112, ¶ 1, the words "written description" hardly prescribe a standard that requires nucleotide-by-nucleotide disclosure.

Under the correct written description test, one of skill in the art would have recognized that the '525 patent in *Lilly* had no new matter or priority problems. In terms of the statutory test for adequacy of disclosure, the patent disclosure undoubtedly warranted rejection for lack of enablement. Under the *In re Wands* test for enablement, 858 F.2d 731, 8 USPQ2d 1400 (Fed.Cir.1988), the inventor certainly did not show one of skill in the art how to make human insulin cDNA. [FN7] Moreover the patent claimed vertebrate insulin cDNA a category ranging from fish to humans again claims whose scope far exceeds the patent's enabling disclosures. In fact, the patent disclosure only revealed that the inventor had enabled cloning of rat insulin. Instead of invalidating under the statutory test for adequacy of disclosure, i.e., enablement, the *Lilly* court purported to create a new doctrine for adequacy of disclosure that it labeled incorrectly "written description." As noted, from its creation through thirty years of application, WD had never been a free-standing substitute for enablement.

> FN7. U.S. Pat. No. 4,652,525, the patent at issue in *Lilly,* was filed in 1983, but claimed priority to a parent filed in 1977. In 1977, biotechnology was still in its infancy. In fact, the Maxam and Gilbert method of sequencing DNA was just published in 1977. Cloning in that era was, at a minimum, unpredictable and would have required vast amounts of experimentation to accomplish. Therefore, the patent's prophetic disclosure of human insulin cDNA hardly enabled its production as claimed. Instead of pursuing this obvious avenue of rejection, the Federal Circuit reached out beyond the statute and the case law to create a new general disclosure test.

Although it should not be necessary, a brief defense of the statutory standard for adequate disclosure shows the flaws of the new form of WD. Enablement already requires inventors to disclose how to *make* (reproduce, replicate, manufacture) and how to *use* the invention (by definition rendering it a "useful art"). Therefore, ***981** because the competitor can make the invention, it can then acquire the DNA sequence or any other characteristic whenever it desires. Meantime the competitor can use, exploit, commercialize (outside the patent term) or improve upon and design around (within the patent term) as much of the invention as it cares to make. In other words, the statutory standard for sufficiency of disclosure serves masterfully the values of the patent system.

Even after *Lilly,* the Federal Circuit--in all other WD cases before this *Enzo* case--applied priority principles, declining to assert the doctrine as a general test for adequacy of disclosure. *See, e.g., Union Oil Co. of Cal. v. Atlantic Richfield Co.,* 208 F.3d 989, 54 USPQ2d 1227 (Fed.Cir.2000). One of those opinions analyzes WD with particular care:

> The written description requirement and its corollary, the new matter prohibition of 35 U.S.C. § 132, both serve to ensure that the patent applicant was in full possession of the claimed subject matter on the application filing date. When the applicant adds a claim or otherwise amends his specification after the original filing date, as Brandon did in this case, the new claims or other added material must find support in the original specification. *TurboCare Div. Of Demag Delaval Turbomachinery Corp. v. General Elec. Co.,* 264 F.3d 1111, 1118, 60 USPQ2d 1017, 1022 (Fed.Cir.2001).

In sum, the written description language has been in the statute since 1870, yet only since 1967 has case law separated it from enablement. The separation itself is not disruptive of the patent system, however, because the doctrine operated solely to police priority. Indeed, with the exception of *Lilly* and this *Enzo* case, this court and its predecessor have only applied the doctrine within the limits of its origin as an "equivalent" or "corollary" of 35 U.S.C. § 132, the new matter section.

Enzo and written description

The record in this case shows that no priority issues remain to invoke WD. The inventor in this case amended the original claims in response to the examiner's request to place the selective hybridization steps in the claims. Thus, the amendments were all narrowing meaning the applicant added no new matter to the claims by amendment. Instead, the applicant copied material from the original specification into the original claims. By definition, this case presents no new matter or priority issues requiring application of the original WD doctrine. The original specification contained all of the subject matter included in the inventor's claims. For this reason, the panel misapplies § 112, ¶ 1 by remanding on the question of WD. *See,* slip op. at 17 ("On remand, the court should consider whether one of skill in the art would find the generically claimed sequences described on the basis of Enzo's disclosure of the hybridization function and an accessible structure, consistent with the PTO Guidelines. If so, the written description requirement would be met."). If any § 112, ¶ 1 questions remain, they are questions of the sufficiency of disclosure, an enablement question. Instead, the panel, relying on *Lilly,* advocates applying WD "regardless whether the claim appears in the original specification and is thus supported by the specification as of the filing date." *Id.* at 18. To the contrary, WD has no such application consistent with the statute and the case law.

Why does this matter?

As both *Lilly* and this case show, the aberrant form of WD requires far more ***982** specific disclosure than enablement. [FN8] Because the *Lilly* application of § 112, ¶ 1 requires a far more demanding disclosure, defendants will have no need to invoke enablement, but will proceed directly to the more demanding *Lilly* § 112, ¶ 1 requirements. Thus, the new breed of WD evident in *Lilly* and this case threatens to further disrupt the patent system by replacing enablement the statutory test for adequate disclosure. *See,* Rai, Arti, "Intellectual Property Rights in Biotechnology: Addressing New Technology" 34 *Wake Forest L.Rev.* 827, 834-35 (Fall, 1999) ("Thus in [*Lilly*] ... the CAFC broke new ground by applying the written description requirement not only to later-filed claims but also to claims filed in the original patent.... [T]he *Lilly* court used the written description requirement as a type of elevated enablement requirement."); Mueller, Janice M., "The Evolving Application of the Written Description Requirement to Biotechnological Inventions" 13 *Berkeley Tech. L.J.* 615, 617 (Spring 1998) ("The *Lilly* decision establishes uniquely rigorous rules for the description of biotechnological subject matter that significantly contort written description doctrine away from its historic origins and policy grounding. The *Lilly* court elevate[s] written description to an effective 'super enablement' standard....").

> FN8. "Conflicts in Federal Circuit Patent Law Decisions," *The Federal Circuit Bar Journal,* Vol. 11, no. 3, p. 723, chronicles this circuit's primary conflicts. Listed first as the leading conflict is "I. The Written Description Requirement of § 112, First Paragraph." *Id.* at 725-34. The article notes: "[T]he Federal Circuit has not provided clear and consistent rules for determining precisely what type of disclosure is sufficient to comply with the § 112 written description requirement." *Id.* at 725. The article then notes three separate tests for measuring compliance with § 112, ¶ 1. For instance, "[t]he strictest approach requires the written description to delineate all of the claimed elements." *Id.*

Furthermore, the Supreme Court repeatedly cautioned against the disruption of the settled expectations of the inventing community. *Festo,* 122 S.Ct. at 1841 ("The responsibility for changing [settled law] rests with Congress.... Fundamental alterations in these rules risk destroying the legitimate expectations of inventors in their property."). *Lilly* and now this case change the application of the WD test and "up the ante" for disclosure a situation inventors might have addressed if they could have foreseen that this court would disrupt settled disclosure principles. At this point, however, those inventors have no way to change patents that comply with enablement disclosure, but not the stiffer demands of *Lilly.*

Replacement of enablement doctrines with an ill-defined general disclosure doctrine of WD imperils the integrity of the patent system. Enablement, arguably the most important patent doctrine after obviousness, has many important applications. Beyond mere adequacy of disclosure, it serves as the line of demarcation between the visionary theorist (adds nothing to the useful arts) and the visionary pioneer (contributes to the useful arts), *see, e.g.,*

Gould v. Hellwarth, 472 F.2d 1383, 176 USPQ 515 (CCPA 1973), and also serves to limit claim scope thus demarking the boundary between pioneer inventions and patentable improvements, *see, e.g., In re Wright*, 999 F.2d 1557 (Fed.Cir.1993). The WD possession test cannot perform these functions. Professor Janis explains that WD provides a blunt tool to measure the sufficiency of disclosure:

> Today, however the written description requirement enjoys a prominence wholly out of proportion to its humble origins.
> ....
> ***983** Recent efforts to elaborate the 'possession' standard both confirm the substantial redundancy of the enablement and written description requirements ....
> ....
> [T]he written description requirement is a threat to the coherence of disclosure doctrines....

Janis, Mark D., "On Courts Herding Cats: Contending with the 'Written Description' Requirement (and Other Unruly Patent Disclosure Doctrines)" 2 *Wash. U.J.L. & Pol'y* 55, 60, 70, 83 (2000).

Professors Rai, Mueller, and Wegner, among others, agree with Professor Janis's assessment. Rai, Mueller, *supra;* Wegner, Harold C., "An Enzo White Paper: A New Judicial Standard for a Biotechnology 'Written Description' Under 35 U.S.C. § 112, ¶ 1" 1 *J. Marshall Rev. Intell. Prop. L.* 254, 263 (2002) (recognizing "there may very well be problems with the scope of enablement in the facts of the *Enzo* case," but written description would not apply to "original claims.").

For biotech inventions, according to the *Lilly* standard, § 112, ¶ 1 requires a precise listing of the DNA sequence nucleotide-by-nucleotide. Enablement, on the other hand, requires that the specification show one of skill in the art how to acquire that sequence on their own. As a test for biotech claims without priority issues, WD may well jeopardize a sizeable percentage of claims filed before the *Lilly* departure in 1997. These patents had no notice of a change in the statutory standard for disclosure. Moreover the *Lilly/Enzo* rule prejudices university or small inventors who do not have the expensive and time-consuming resources to process every new biotechnological invention to extract its nucleotide sequence. *See,* Mueller, *supra* at 617 ("*Lilly* ... will likely chill development."); Sampson, Margaret, "The Evolution of the Enablement and Written Description Requirements Under 35 U.S.C. § 112 in the Area of Biotechnology." *15 Berkeley Tech. L.J.* 1233, 1262 (Fall 2000) ("The primary argument against the Federal Circuit's heightened written description requirement for biotechnological invention is that ... it also 'reduces incentives to invest in innovation by depriving potential patentees of the opportunity to fully benefit from their research.' ").

Saving the obvious for last, *Lilly* and this case really cannot depart from decades of established case law on § 112, ¶ 1. Even the court's decision to issue this improved version of *Enzo* without correcting all the problems does not indicate any acceptance of written description as a general disclosure doctrine for all claims regardless of priority issues. *Lilly* and this case are panel cases and cannot override the statute that makes enablement the general disclosure doctrine and the vast body of prior case law limiting WD to its original purpose. Sadly, however, this case will perpetuate the confusion.

Conclusion

Written description a part of the Patent Act since 1870 has taken on a life separate from its statutory context only since 1967. As long as WD applied only for the reasons that occasioned its judicial creation, it did not disrupt the rest of the Patent Act. Two recent cases, however, this case and the 1997 *Lilly* case, have purported to create a new disclosure doctrine that supplants enablement. Although this court declines to take this occasion to correct those dalliances, the origin and purpose of both § 112, ¶ 1 doctrines serve notice that neither *Lilly* nor this case properly applies the otherwise orderly disclosure doctrines.

***984** APPENDIX

CCPA

1. *In re Ruschig*, 54 C.C.P.A. 1551, 379 F.2d 990 (1967). "These claims were under rejection by reason of one-year statutory bars which could be overcome only by reliance on the filing date of the present *parent* application which gave rise to the question whether the application contained support for the claims." *Id.* at 991.

I. In re Ahlbrecht, 58 C.C.P.A. 848, 435 F.2d 908 (1971). "[T]he parties disagree as to whether the disclosure in the *earlier* application is sufficient under the first paragraph of 35 U.S.C. § 112 to support the invention claimed in claim 7." *Id.* at 909.

3. *Fields v. Conover*, 58 C.C.P.A. 1366, 443 F.2d 1386 (1971). "[E]ven when considered ... it falls far

short ... of the 'full, clear, concise, and exact' written description which we have said is necessary to support *subsequently* added claims." *Id.* at 1392.

4. *In re Smith*, 59 C.C.P.A. 1025, 458 F.2d 1389 (1972). "[A]ppellant has no basis on which the disclosure in the 1947 application may be treated as a description of the subject matter *now* claimed." *Id.* at 1394.

5. *In re Gardner*, 475 F.2d 1389 (CCPA 1973). "Claim 2, which apparently was an original claim, in itself constituted a description in the original disclosure equivalent in scope and identical in language to the total subject matter now being claimed." *Id.* at 1391.

6. *In re Smith*, 481 F.2d 910 (CCPA 1973). "Satisfaction of the description requirement insures that subject matter presented in the form of a claim *subsequent* to the filing date of the application was sufficiently disclosed at the time of filing so that prima facie date of invention can fairly be held to be the filing date of the application.... The specification as originally filed must convey clearly to those skilled in the art the information that the applicant has invented the specific subject matter *later* claimed." *Id.* at 914.

7. *In re Mott*, 539 F.2d 1291 (CCPA 1976). "The issue under this heading is whether appellant's specification, construed in light of the knowledge of those skilled in this art, contains a written description of the subject matter of claims 42, 44, and 46." (Claims 42, 44 and 46 were claims copied from the Taylor patent and put in the application by *amendment.*) *Id.* at 1296.

8. *In re Wertheim*, 541 F.2d 257 (CCPA 1976). "The dispositive issue under this heading is whether appellants' parent and Swiss applications comply with 35 U.S.C. § 112, first paragraph, including the description requirement, as to the subject matter of these claims. If they do, these claims are entitled to the filing dates of the *parent* application ... [A] right of foreign *priority* in appellants' Swiss application will antedate Pfluger 1966 and remove it as prior art against the claims." *Id.* at 261. "The function of the description requirement is to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter *later* claimed by him." *Id.* at 262.

9. *In re Blaser*, 556 F.2d 534 (CCPA 1977). "The function of the description requirement is to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter *later* claimed by him." *Id.* at 537 (quoting *In re Wertheim*, 541 F.2d at 262).

10. *In re Barker*, 559 F.2d 588 (CCPA 1977). "We can find no indication in the specification or claims as originally filed that appellants invented the subject matter *now* claimed." *Id.* at 593.

***985** 11. *In re Driscoll*, 562 F.2d 1245 (CCPA 1977). "Appellant does not dispute that the appealed claim is anticipated by the Belgian patent if the present application is not entitled to the *earlier* filing date of S.N. 782,756. Consequently, the sole issue with respect to this aspect of the appeal is whether the disclosure of S.N. 782,756 described the subject matter of claim 13. In resolving this issue, we must view the disclosure of the *earlier* filed application as would a person skilled in the art and determine whether it reasonably conveys the information that as of the filing date thereof appellant had possession of the class of 5-alkylsulfonyl-1, 3, 4- thiadiazole ureas defined in claim 13." *Id.* at 1248-49.

12. *In re Edwards*, 568 F.2d 1349 (CCPA 1978). "The dispositive issue is whether appellants' *parent* application, serial No. 682,560, filed November 13, 1967, complies with the written description requirement of 35 U.S.C. § 112, first paragraph, vis-à-vis the subject matter of the appealed claim; if it does, then the claim is entitled to the filing date of the *parent* application under 35 U.S.C. § 120." *Id.* at 1351.

13. *In re Herschler*, 591 F.2d 693 (CCPA 1979). "[A]ppellant concedes that the substance of this rejection is proper if the court finds either the *great-grandparent* application lacks a written description of the *instant* invention." *Id.* at 699.

14. *In re Rasmussen*, 650 F.2d 1212 (CCPA 1981). "The proper basis for rejection of a claim amended to recite elements thought to be without support in the original disclosure, therefore, is § 112, first paragraph, not § 132. The latter section prohibits addition of new matter to the original disclosure. It is properly employed as a basis for objection to amendments to the abstract, specifications, or drawings attempting to add new disclosure to that originally presented." *Id.* at 1214-15.

Federal Circuit

1. *In re Kaslow*, 707 F.2d 1366 (Fed.Cir.1983).

"The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the *later* claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language." *Id.* at 1375.

2. *Ralston Purina Co. v. Far-Mar-Co.*, 772 F.2d 1570 (Fed.Cir.1985). "[T]he test for sufficiency of support in a *parent* application is whether the disclosure of the application relied upon 'reasonably conveys to the artisan that the inventor had possession at that time of the *later* claimed subject matter.' " *Id.* at 1575.

3. *Kennecott Corp. v. Kyocera Int'l., Inc.*, 835 F.2d 1419 (Fed.Cir.1987). "The incorporation of the requirements of section 112 into section 120 ensures that the inventor had possession of the *later*-claimed invention on the filing date of the *earlier* application." *Id.* at 1421.

4. *Utter v. Hiraga*, 845 F.2d 993 (Fed.Cir.1988). "Hiraga's Japanese specification complies with the written description requirement of Section 112 if 'the disclosure of the application as originally filed reasonably conveys to the artisan that [Hiraga] had possession at that time of the *later* claimed ['068 interference count] subject matter.' " *Id.* at 999.

5. *Bigham v. Godtfredsen*, 857 F.2d 1415 (Fed.Cir.1988). "This requirement applies to *priority* claims under 35 U.S.C. § 119.... The test is whether the disclosure of 'halogen,' exemplified by chloro, meets the requirements of § 112 as a written ***986** description of the bromo and iodo species in the context of the specific invention at issue." *Id.* at 1417.

6. *United States Steel Corp. v. Phillips Petroleum Co.*, 865 F.2d 1247 (Fed.Cir.1989). "In the context of section 120, in this case, focusing on the filing date requires that the claim of the 851 patent be treated as though it were filed in 1953. Only if that claim would at that time have been correctly rejected for lack of support in the 1953 specification may the patentee be denied use of section 120 to *predate* the intervening reference to the '300 patent." *Id.* at 1251.

7. *In re Wright*, 866 F.2d 422 (Fed.Cir.1989). "When the scope of a claim has been changed by amendment in such a way as to justify an assertion that it is directed to a different invention than was the original claim, it is proper to inquire whether the *newly* claimed subject matter was described in the patent application when filed as the invention of the applicant. That is the essence of the so-called 'description requirement' of § 112, first paragraph." *Id.* at 424.

8. *Chester v. Miller*, 906 F.2d 1574 (Fed.Cir.1990). "[T]he EIC simply found that the '280 reference (*parent*) did not support the '122 application claims because as to them it failed to meet the written description requirement." *Id.* at 1577.

9. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555 (Fed.Cir.1991). "The purpose and applicability of the 'written description' requirement ... insure[ ] that subject matter presented in the form of a claim *subsequent* to the filing date of the application was sufficiently disclosed at the time of filing so that the prima facie date of invention can fairly be held to be the filing date of the application." *Id.* at 1562.

10. *In re Hayes Microcomputer Products, Inc.*, 982 F.2d 1527 (Fed.Cir.1992). "The test for sufficiency of support in a *parent* application is whether the disclosure of the application relied upon 'reasonably conveys to the artisan that the inventor had possession at that time of the *later* claimed subject matter.' " *Id.* at 1532.

11. *Fiers v. Revel*, 984 F.2d 1164 (Fed.Cir.1993). "Revel bears the burden of proving entitlement to the benefit of his *earlier*-filed Israeli application date.... Revel must prove that his application meets the requirements of 35 U.S.C. § 112, first paragraph." *Id.* at 1169.

12. *Mendenhall v. Cedarapids*, 5 F.3d 1557 (Fed.Cir.1993). "Mr. Mendenhall himself testified that he did not have any invention directed to introducing virgin aggregate and RAP as specified in the '904 claims until December 1977, and there is no description of that invention in the parent or grandparent applications.... A patentee cannot obtain the benefit of the filing date of an *earlier* application where the claims in issue could not have been made in the *earlier* application." *Id.* at 1565-66.

13. *Eiselstein v. Frank*, 52 F.3d 1035 (Fed.Cir.1995). "In order to determine whether a prior application meets the 'written description' requirement with respect to *later*-filed claims, the prior application .... The test is whether the disclosure of the application relied upon reasonably conveys to a person skilled in the art that the inventor had possession of the claimed subject matter at the time of the *earlier* filing date."

Id. at 1038-39.

14. *In re Alton*, 76 F.3d 1168 (Fed.Cir.1996). "The adequate written description requirement ... serves 'to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter *later* claimed by him'." *Id.* at 1172.

***987** 15. *Kolmes v. World Fibers Corp.*, 107 F.3d 1534 (Fed.Cir.1997). "The question raised here is whether the claims *added* by the preliminary amendment to the 1992 *continuation* application find adequate support in the 1990 application sufficient to meet the description requirement of section 112, ¶ 1." *Id.* at 1539.

16. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565 (Fed.Cir.1997). "[A] *prior* application itself must describe an invention, and do so in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought." *Id.* at 1572.

After LILLY

1. *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473 (Fed.Cir.1998). "Accordingly, his original disclosure serves to limit the permissible breadth of his *later*-drafted claims." *Id.* at 1479.

2. *Tronzo v. Biomet, Inc.*, 156 F.3d 1154 (Fed.Cir.1998). "For a claim in a *later*-filed application to be entitled to the filing date of an *earlier* application under 35 U.S.C. sec. 120, the *earlier* application must comply with the written description requirement of 35 U.S.C. section 112, ¶ 1." *Id.* at 1158.

3. *Union Oil Co., of Cal. v. Atlantic Richfield Co.*, 208 F.3d 989 (Fed.Cir.2000). "However, neither the Patent Act nor the case law of this court requires such detailed disclosure.... Rather the Patent Act and this court's case law require only sufficient description to show one of skill in the refining art that the inventor possessed the claimed invention at the time of filing." *Id.* at 997.

4. *Reiffin v. Microsoft Corp.*, 214 F.3d 1342 (Fed.Cir.2000). "In accordance with § 120, claims to subject matter in a *later*-filed application not supported by an ancestor application in terms of § 112 ¶ 1 are not invalidated; they simply do not receive the benefit of the *earlier* application's filing date." *Id.* at 1346.

5. *Lampi Corp. v. American Power Products, Inc.*, 228 F.3d 1365 (Fed.Cir.2000). "For a claim in a *later*-filed application to be entitled to the filing date of an *earlier* application under 35 U.S.C. 120, the *earlier* application must comply with the requirement of 35 U.S.C. § 112, ¶ 1." *Id.* at 1377. "The requirement is met if 'the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession at that time of the *later* claimed subject matter.' " *Id.* at 1378.

6. *Purdue Pharma L.P. v. Faulding, Inc.*, 230 F.3d 1320 (Fed.Cir.2000). "[W]e conclude that the district court did not commit clear error in finding that nothing in the '688 application 'necessarily' ... described the *later* claimed subject matter of the '360 patent." *Id.* at 1327.

7. *TurboCare Div. Of Demag Delaval Turbomachinery Corp. v. General Elec. Co.*, 264 F.3d 1111 (Fed.Cir.2001). "The written description requirement and its corollary, the new matter prohibition of 35 U.S.C. § 132, both serve to ensure that the patent applicant was in full possession of the claimed subject matter on the application filing date. When the applicant adds a claim or otherwise amends his specification after the original filing date, as Brandon did in this case, the new claims or other added material must find support in the original specification." *Id.* at 1118.

LINN, Circuit Judge, with whom RADER and GAJARSA, Circuit Judges, join, dissenting from the court's decision not to the hear the case en banc.

I am in agreement with much of the panel's reasoning in the revised opinion, ***988** but part company with the panel's treatment of written description and enablement issues, most notably in the text dealing with the *in ipsis verbis* issue.

With all due respect, the panel opinion in my view conflates and perpetuates the confusion our precedent has engendered between written description as a separate requirement ("possession of the invention")--an issue relevant to priority--and enablement--an issue relevant to the sufficiency of the disclosure. The notion of having to show "possession of the invention" was discussed in *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed.Cir.1991) and other cases from our court as a convenient way to measure or test entitlement of later filed claims to an earlier priority date. It was not and

should not be a test for sufficiency of disclosure, per se. It should have no place in and does not aid in the disposition of cases where the claims in question are part of the original disclosure. In those cases, entitlement to the filing date is inherent in that the claims themselves--having been filed as part of the original application--provide their own written description.

35 U.S.C. § 112 requires a written description of the invention, but the measure of the sufficiency of that written description in meeting the conditions of patentability in paragraph 1 of that statute, either by reference to a microorganism deposit or in terms *in ipsis verbis* with the language of the claims, should depend solely on whether it enables any person skilled in the art to which the invention pertains to make and use the claimed invention. Where priority is not an issue, as in the present case, the focus once a written description has been found should be on whether the description meets the enablement requirement. Satisfaction of the "possession of the invention" test simply is not relevant.

The question presented by 35 U.S.C. § 112, paragraph 1, is not, "Does the written description disclose what the invention is, or does it merely describe what it does?" The question is, "Does the written description describe the invention recited and described in the claims--themselves part of the specification--in terms that are sufficient to enable one of skill in the art to make and use the claimed invention?" That is the mandate of the statute and is all our precedent, prior to *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed.Cir.1997) and the present case, demand. For original claims, where priority is not an issue, the notion of possession of the invention is not germane, the claim itself evidencing possession of the invention as of the filing date. In the panel opinion, the discussion of the *in ipsis verbis* issue properly addresses enablement issues but does so in words not of enablement but of "possession of the invention." This conflates the two unrelated issues, elevates "possession" to the posture of a statutory test of patentability--which it is not--and fosters further confusion in what is already a confusing area of our precedent.

The U.S. Patent and Trademark Office ("PTO") aptly states the reason why this case should be taken en banc: "[a]lthough this Court has addressed the 'written description' requirement of section 112 on a number of occasions, its decisions have not taken a clear and uniform position regarding the purpose and meaning of the requirement." PTO amicus brief at 4.

This is an area of law that is of significant importance to the biotech industry and affects how patent applications are drafted, prosecuted and will be enforced in this and other areas of emerging technology. When patent attorneys set out to write patent applications, they do so for an educated audience--those skilled in the ***989** art--and attempt to describe the invention in a way that enables those of ordinary skill to make and use the invention as claimed. Before the decision in *Lilly*, the practicing bar had accepted and found workable the notion elucidated in our precedent that § 112 requires a written description sufficient to enable one of ordinary skill in the art to make and use the claimed invention--i.e., enablement. *Lilly* changed the landscape and engendered the debate the panel opinion in this case perpetuates.

Some have praised *Lilly* for maintaining the integrity of patent disclosures and for curbing patent filings for inventions that have not yet been made but are just nascent ideas. Others have been sharply critical of *Lilly*. The debate is well framed by the panel opinion and the contemporaneous dissent of Judge Rader. Those opinions highlight the uncertainty this issue raises in how inventions are protected, in how the PTO discharges its responsibilities, and in how business is conducted in emerging fields of law. These uncertainties will be left unresolved until we clarify this en banc. The issue is important, is ripe for us to consider, and deserves to be clarified, one way or the other. For these reasons, I respectfully dissent from the court's declining to consider this case en banc.

323 F.3d 956, 63 U.S.P.Q.2d 1609

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Only the Westlaw citation is currently available.

United States Court of Appeals,
Federal Circuit.

Randolph J. NOELLE, Appellant,
v.
Seth LEDERMAN, Leonard Chess, and Michael J. Yellin, Appellees.

No. 02-1187.

Jan. 20, 2004.

Background: Applicant for patent related to monoclonal antibody sought judicial review of decision by Board of Patent Appeals and Interferences, 2001 WL 1285978, that certain claims of application were unpatentable as anticipated by prior art patent, and that there was no interference in fact between application and prior art patent.

Holdings: The Court of Appeals, Gajarsa, Circuit Judge, held that:
(1) evidence supported finding that specification in parent patent application for murine antibody did not satisfy written description requirement for subsequent application's human and genus antibody claims, and
(2) evidence supported finding of noninterference.
Affirmed.

[1] **Patents** 314(5)

291k314(5) Most Cited Cases

Whether patent specification complies with written description requirement is question of fact. 35 U.S.C.A. § 112, ¶ 1.

[2] **Patents** 110
291k110 Most Cited Cases

Test to determine if earlier filed patent application satisfies written description requirement for claims in later application, and thus whether later application is to receive benefit of earlier application's filing date, is whether person of ordinary skill in art would recognize that applicant possessed what is claimed in later filed application as of filing date of earlier filed application. 35 U.S.C.A. § 112, ¶ 1.

[3] **Patents** 110
291k110 Most Cited Cases

Earlier patent application that describes later-claimed genetic material only by statement of function or result may be insufficient to meet written description requirement, for purpose of determining whether later claims are entitled to earlier application's filing date. 35 U.S.C.A. § 112, ¶ 1.

[4] **Patents** 99
291k99 Most Cited Cases

Patent specification's characterization of antibody by its binding affinity to antigen satisfies written description requirement, so long as antigen is adequately characterized, either by its structure, formula, chemical name, or physical properties, or by depositing protein in public depository. 35 U.S.C.A. § 112, ¶ 1.

[5] **Patents** 110
291k110 Most Cited Cases

Evidence supported finding that specification in parent patent application for murine antibody did not satisfy written description requirement for subsequent application's human and genus antibody claims, and thus that subsequent application was not entitled to parent's earlier filing date; parent application failed to disclose structural elements either of human antibody or of antigen to which it had binding affinity. 35 U.S.C.A. § 112, ¶ 1.

[6] **Patents** 106(1)
291k106(1) Most Cited Cases

Under "two-way test," patents interfere if each would be anticipated or obvious if other, in turn, was presumed to be prior art. 37 C.F.R. § 1.601(n).

[7] **Patents** 106(3)
291k106(3) Most Cited Cases

Evidence supported finding that patent for human form of antibody and patent application for mouse form of same antibody did not interfere in fact; there was expert testimony that skilled artisan in possession of either invention would not have had reasonable likelihood of success in obtaining other invention without undue experimentation. 37 C.F.R. § 1.601(n).

[8] Patents 106(1)
291k106(1) Most Cited Cases

Method of isolating human antigen, found in specification of patent application claiming murine antibody, could not be considered when determining whether there was interference in fact between application and patent for human antibody; test for interference compared parties' inventions, which were only found in claims. 37 C.F.R. § 1.601(n).

Patents 328(2)
291k328(2) Most Cited Cases

5,474,771, 5,961,974. Cited as Prior Art.
Appealed from United States Patent and Trademark Office, Board of Patent Appeals and Interferences.

E. Anthony Figg, Rothwell, Figg, Ernst & Manbeck, of Washington, DC, argued for appellant. With him on the brief was Glenn E. Karta.

James F. Haley, Jr., Fish & Neave, of New York, NY, argued for appellees. With him on the brief were Margaret A. Pierri and Jane T. Gunnison. Of counsel on the brief was John P. White, Cooper & Dunham LLP, of New York, NY. Of counsel was Stanley Den-Kua Liang, Fish & Neave.

Before CLEVENGER, BRYSON, and GAJARSA, Circuit Judges.

GAJARSA, Circuit Judge.

*1 This is an appeal from an interference proceeding involving the claims of United States Patent Application Serial No. 08/742,480 (the "'480 application") and United States Patent No. 5,474,771 (the "'771 patent"). Randolph J. Noelle ("Noelle") is the inventor named on the '480 application. Seth Lederman, Leonard Chess, and Michael J. Yellin (collectively "Lederman") are the inventors named on the '771 patent. Noelle appeals the decision of the United States Patent and Trademark Office, Board of Patent Appeals and Interferences ("Board"), finding no interference-in-fact between the '480 application and the '771 patent and rejecting claims 51, 52, 53, 56, 59, and 60 of the '480 application pursuant to 35 U.S.C. § 102(b) (2000). *Noelle v. Lederman*, Interference No. 104,415 (Bd. Pat.App. & Int. Oct. 19, 2001). Because the decision of the Board is supported by substantial evidence and is not contrary to law, we affirm.

BACKGROUND

A. *Antibodies*

This case relates to antibodies and their role in the immune response system. A vertebrate's immune system serves to identify and destroy foreign invading organisms and neutralize the toxic molecules they produce. Antibodies, which are proteins also referred to as immunoglobulins ("Ig"), serve to designate foreign particles, broadly referred to as antigens, for destruction by other components of the immune system such as lymphocytes. [FN1] Lymphocytes, otherwise known as white blood cells, produce antibodies and destroy antigens. T-cells and B-cells are the two types of lymphocytes needed for antibody production. One specific type of T-cell is the helper T-cell. Helper T-cells recognize antigens and then induce B-cells to produce antibodies through a series of events. First the helper T-cell is activated after it recognizes an antigen. Once activated, the helper T-cell activates the B-cell by a combination of binding with the B-cell and secreting signaling molecules. Once the B-cell is activated, it differentiates, [FN2] proliferates, and produces antibodies specific to a particular antigen. The antibodies then circulate in the bloodstream and permeate other bodily fluids, where they bind to the antigen, thereby flagging it for destruction.

The present interference involves competing claims to an antibody ("CD40CR antibody") that represses the cell-to-cell signaling interaction between helper T-cells and B-cells. CD40CR antigen [FN3] is found on activated, but not resting, helper T-cells. CD40CR antigen acts as a "key" to unlock a protein ("CD40") located on the surface of resting B-cells. Once CD40CR antigen and CD40 bind, the B-cell begins down the pathway to differentiation, proliferation, and antibody production. The CD40CR antibody binds to the CD40CR antigen located on the T-cell surface, thereby inhibiting its ability to bind to the CD40 receptor located on the resting B-cell. B-cells cannot then become activated, thereby preventing the B-cell from producing antibodies. CD40CR antibodies are useful for treating a hyperactive immune system that causes allergic reactions and autoimmune diseases.

B. *The Interference*

*2 Noelle's '480 application was filed November 1,

1996. The '480 application is a continuation of application Serial No. 08/338,975 ("the '975 application"), filed November 14, 1994, which is in turn a continuation of application Serial No. 07/835,799 ("the '799 application"), filed on February 14, 1992. The claims of Noelle's '480 application are directed to the genus, murine ("mouse"), chimeric ("hybrid"), humanized, and human forms of the CD40CR monoclonal antibody. Noelle also claims the hybridomal [FN4] cell lines that produce the CD40CR antibody.

Lederman's '771 issued patent has an effective filing date of November 15, 1991. Lederman's '771 patent describes and claims the human form of CD40CR monoclonal antibody (the "5c8 antibody"). The 5c8 antibody binds to "the 5c8 antigen located on the surface of activated T cells and thereby inhibits T cell activation of B cells." Also, Lederman claims a hybridomal cell line created to produce monoclonal antibody 5c8.

On September 3, 1999, an interference was declared by the United States Patent and Trademark Office ("USPTO") between the issued claims of Lederman's '771 patent and Noelle's '480 application. Noelle was designated the junior party and Lederman was designated the senior party based on their effective filing dates. The USPTO established only one count in the interference. The count reads as follows:

> The monoclonal antibody of claim 1 of 5,474,771 or the monoclonal antibody of claim 42 or claim 51 of 08/742,480.

Claim 1 of Lederman's '771 patent reads as follows:

> A monoclonal antibody, which specifically binds and forms a complex with the 5c8 antigen located on the surface of activated T cells and thereby inhibits T cell activation of B cells, the 5c8 antigen being an antigen to which monoclonal antibody 5c8 (ATCC Accession No. HB 10916) specifically binds.

Claim 42 of Noelle's '480 application reads as follows:

> A monoclonal antibody or fragment thereof which specifically binds to an antigen expressed on activated T cells, wherein said antigen is specifically bound by the monoclonal antibody secreted by hybridoma MR1 which hybridoma has been deposited and accorded ATCC Accession No. HB 11048.

Claim 51 of Noelle's '480 application reads as follows:

> A monoclonal antibody or fragment thereof which specifically binds CD40CR.

Claim 52 of Noelle's '480 application reads as follows:

> The monoclonal antibody or fragment of Claim 51, wherein said CD40CR is expressed by activated human T cells.

For sake of the simplicity, Claim 1 of Lederman's '771 patent and Claim 52 of Noelle's '480 application will be referred to as claims to the "human" form of CD40CR antibody. Claims 42 and 51 of Noelle's '480 application will be referred to as claims to the "mouse" and "genus" forms of CD40CR antibody, respectively.

On June 28, 2001 the Board held a hearing to dispose of the parties' preliminary motions. Lederman moved to have Noelle's claims rejected and sought to redefine the count. Likewise, Noelle also sought to have the count redefined. The Board denied Lederman's motions for judgment against Noelle's mouse claims for lack of written description, lack of enablement, and indefiniteness. *See* 35 U.S.C. § 112 (2000). The Board found that Lederman had failed to demonstrate that the mouse claims in Noelle's '480 application failed to comply with 35 U.S.C. § 112, paragraphs (1) and (2), as of November 1, 1996, the date Noelle filed his '480 application. The Board, however, determined that the human and genus claims in Noelle's '480 application failed to comply with the written description requirement pursuant to 35 U.S.C. § 112, paragraph (1), as of February 14, 1992, the date Noelle filed the previous '799 application. The Board made a detailed analysis of this court's precedent pertaining to the doctrine of written description, focusing on the holding from *Regents of the University of California v. Eli Lilly & Co.* that an "adequate written description of a DNA sequence claim requires a precise definition, such as structure, formula, chemical name, or physical properties." 119 F.3d 1559, 1566 (Fed.Cir.1997). The Board analogized the DNA claims from *Regents* to the antibodies in Noelle's application. Accordingly, the Board held that Noelle's claims regarding the genus and human claims from the '480 application lacked written description support in the specification of Noelle's earlier '799 application because Noelle failed to describe any structural features of the human or genus antibodies or antigens. In other words, the Board found that the claims covering the genus and human antibodies constituted new matter because they lacked adequate written description in Noelle's earlier '799 application. The Board did not reject the

claims, but rather denied them the benefit of the earlier filing date of Noelle '799.

*3 Next, the Board addressed the implication of finding a lack of written description for the genus and human claims in Noelle's '480 application. The Board determined that the claims to the human and genus forms of CD40CR antibody in Noelle's '480 application were anticipated by either Lederman '771, which claims priority to U.S. Application 07/792,728, filed November 15, 1991, or Armitage 5,961,974 (the "'974 patent"), which claims priority to U.S. applications 07/783,707 and 07/805,723 filed October 25, 1991, and December 5, 1991, respectively. Noelle had not attempted to distinguish his human and genus claims from the prior art and had conceded that Lederman '771 and Armitage '974 would anticipate those claims if the '480 application were not afforded the earlier filing date of Noelle's '799 application. Thus, the Board found the genus and human claims of Noelle's '480 application to be anticipated under 35 U.S.C. § 102(b) by the two forms of prior art and, as a result, rejected the claims to the human and genus forms of CD40CR antibodies and their respective cell lines pursuant to 37 C.F.R. § 1.641.

On October 19, 2001, the Board ruled on the motions remaining from the previous hearing. The Board had determined in its previous hearing that the deferred motions were essentially requests to decide whether an interference- in-fact existed between the two parties' claims. Lederman then withdrew his pending motions and filed a new motion requesting that the Board find no interference-in-fact.

The Board concluded from the evidence submitted that there was no interference-in-fact. The Board reasoned that a person of ordinary skill in the art lacked a reasonable expectation of success of obtaining the other party's claimed invention given the state of the art at the time. The Board noted three different methods disclosed in Noelle's '480 specification by which a person of ordinary skill in the art could have isolated the human form of the CD40CR antibody given the mouse version of the CD40CR antibody. Dr. Edward A. Clark, Noelle's expert, declared that a person skilled in the art would have had a reasonable expectation of success in isolating human CD40CR antibody by utilizing the methods disclosed in Noelle's specification.

First, Clark testified that human CD40CR antibody could be isolated by immunizing a host with human CD40CR antigen expressing cells or cell lines and selecting the antibody to the CD40CR antigen by functional or competition binding with CD40-Ig. [FN5] Next, Clark suggested methods of making and isolating antibodies using affinity purified human CD40CR antigen. Last, Dr. Clark declared that one skilled in the art could use the mouse CD40CR antibody or CD40-Ig to clone CD40CR antigen DNA using a method known as expression cloning.

The Board found that one skilled in the art would not have had a reasonable expectation of success of isolating human CD40CR antibodies given the mouse form of CD40CR antigen. At the outset, the Board reasoned that any reference to Noelle's own specification as prior art was improper because the specifications underlying the respective claims cannot be considered "prior art" and an interference-in-fact analysis requires the comparison between the parties' claims, not their specifications. *In re Vaeck*, 947 F.2d 488, 493 (Fed.Cir.1991). Nevertheless, the Board refuted the three methods disclosed in Noelle's specification and endorsed by Clark. First, the Board found that the immunization technique found in the prior art would be ineffective because, at the relevant time, one skilled in the art would not have had a reasonable expectation of success of identifying the activated T-cells that produced the required CD40CR antigen or of isolating the antigen itself. Second, the Board found that it would have been "extremely difficult" for a person of ordinary skill in the art to isolate successfully CD40-Ig, which, as Noelle asserted, could then be used to obtain the claimed CD40CR antibodies. Third, the Board cited statements made during the prosecution of Armitage application 07/969,703 for the proposition that a skilled artisan could not have used expression cloning to isolate CD40CR antibody with a reasonable likelihood of success.

*4 Thus, the Board determined that a person of ordinary skill in the art would not have been reasonably likely to isolate human CD40CR antibody given Noelle's claimed invention of mouse CD40CR antibody. As a result, the Board found no interference-in-fact between Noelle's remaining murine CD40CR antibody claim and Lederman's claim to the human form of CD40CR antibody. Noelle timely appealed to this court and we have jurisdiction under 28 U.S.C. § 1295(a)(4)(A) (2000).

DISCUSSION

[1] Whether a specification complies with the written description requirement of 35 U.S.C. § 112, paragraph (1), is a question of fact, *Vas-Cath Inc. v.*

Mahurkar, 935 F.2d 1555, 1562 (Fed.Cir.1991), and is, in appeals from the Board, reviewed under the substantial evidence standard. *In re Gartside*, 203 F.3d 1305, 1315 (Fed.Cir.2000). To apply a substantial evidence standard, this court must "examin[e] the record as a whole, taking into account evidence that both justifies and detracts from an agency's decision." *Id.* at 1312. A reviewing court must ask "whether a reasonable fact finder could have arrived at the agency's decision." *Id.* "[T]he possibility of drawing two inconsistent conclusions from the evidence does not prevent an administrative agency's finding from being supported by substantial evidence." *Id.*

A. *Entitlement to Priority*

[2][3] The written description requirement has been defined many times by this court, but perhaps most clearly in *Vas-Cath*. The court held as follows:

> 35 U.S.C. § 112, first paragraph, requires a "written description of the invention" which is separate and distinct from the enablement requirement. The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the "written description" inquiry, *whatever is now claimed.*

Vas-Cath, 935 F.2d at 1563-64 (emphasis in original). Thus, the test to determine if an application is to receive the benefit of an earlier filed application is whether a person of ordinary skill in the art would recognize that the applicant possessed what is claimed in the later filed application as of the filing date of the earlier filed application. An earlier application that describes later-claimed genetic material only by a statement of function or result may be insufficient to meet the written description requirement. *See Regents*, 119 F.3d at 1566. This court has held that a description of DNA " 'requires a precise definition, such as by structure, formula, chemical name, or physical properties,' not a mere wish or plan for obtaining the claimed chemical invention." *Id.* (quoting *Fiers v. Revel*, 984 F.2d 1164, 1170 (Fed.Cir.1993)). Therefore, this court has held that statements in the specification describing the functional characteristics of a DNA molecule or methods of its isolation do not adequately describe a particular claimed DNA sequence. Instead "an adequate written description of DNA requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself." *Id.* at 1566-67 (quoting *Fiers*, 984 F.2d at 1171). It should be noted, however, that this court in *Vas-Cath* warned that each case involving the issue of written description, "must be decided on its own facts. Thus, the precedential value of cases in this area is extremely limited." *Vas-Cath*, 935 F.2d at 1562 (quoting *In re Driscoll*, 562 F.2d 1245, 1250 (C.C.P.A.1977)).

***5** Indeed, the court in *Enzo Biochem v. Gen-Probe, Inc.*, 323 F.3d 956, 964 (Fed.Cir.2002) ("*Enzo Biochem II* "), stated that "the written description requirement would be met for all of the claims [of the patent at issue] if the functional characteristic of [the claimed invention was] coupled with a disclosed correlation between that function and a structure that is sufficiently known or disclosed." Also, the court held that one might comply with the written description requirement by depositing the biological material with a public depository such as the American Type Culture Collection ("ATCC"). *Id.* at 970. The court proffered an example of an invention successfully described by its functional characteristics. The court stated:

> For example, the PTO would find compliance with 112, paragraph 1, for a claim to an isolated antibody capable of binding to antigen X, notwithstanding the functional definition of the antibody, in light of the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature.

Id. The court adopted the USPTO Guidelines as persuasive authority for the proposition that a claim directed to "any antibody which is capable of binding to antigen X" would have sufficient support in a written description that disclosed "*fully characterized* antigens." Synopsis of Application of Written Description Guidelines, at 60, *available at* http://www.uspto.gov/web/menu/written.pdf (last visited Jan. 16, 2003) (emphasis added).

[4] Therefore, based on our past precedent, as long as an applicant has disclosed a "*fully characterized* antigen," either by its structure, formula, chemical name, or physical properties, or by depositing the protein in a public depository, the applicant can then claim an antibody by its binding affinity to that described antigen.

[5] Noelle did not provide sufficient support for the claims to the human CD40CR antibody in his '480 application because Noelle failed to disclose the

structural elements of human CD40CR antibody or antigen in his earlier '799 application. Noelle argues that because antibodies are defined by their binding affinity to antigens, not their physical structure, he sufficiently described human CD40CR antibody by stating that it binds to human CD40CR antigen. Noelle cites *Enzo Biochem II* for this proposition. This argument fails, however, because Noelle did not sufficiently describe the human CD40CR antigen at the time of the filing of the '799 patent application. In fact, Noelle only described the mouse antigen when he claimed the mouse, human, and genus forms of CD40CR antibodies by citing to the ATCC number of the hybridoma secreting the mouse CD40CR antibody. If Noelle had sufficiently described the human form of CD40CR antigen, he could have claimed its antibody by simply stating its binding affinity for the "fully characterized" antigen. Noelle did not describe human CD40CR antigen. Therefore, Noelle attempted to define an unknown by its binding affinity to another unknown. As a result, Noelle's claims to human forms of CD40CR antibody found in his '480 application cannot gain the benefit of the earlier filing date of his '799 patent application.

***6** Moreover, Noelle cannot claim the genus form of CD40CR antibody by simply describing mouse CD40CR antigen. Noelle cites *Staehelin v. Secher*, 24 U.S.P.Q.2d 1513, 1519 (Bd. Pat.App. & Int. Sept. 28, 1992), as support for his argument that he has rights to the genus form of CD40CR antibody. In *Staehelin,* Dr. Secher had developed a hybridoma that produced a monoclonal antibody targeted to an antigen unavailable in pure form. *Id.* The antigen was human leukocyte interferon. *Id.* In Secher's foreign application, he had reported the isolation of a hybridoma-secreting antibody to human leukocyte interferon. *Id.* In his subsequent U.S. application, Secher claimed the genus form of the antibody. *Id.* at 1520. The Board held, "Secher's disclosure ... would have reasonably conveyed to a person possessing ordinary skill in the art that Secher possessed the genus later claimed by them in their U.S. application in the sense of 35 U.S.C. 112, first paragraph." *Id.* The Board held it is not necessary to describe the exact details for preparing every species within the genus in order to claim the genus. *Id.* (citing *Utter v. Hiraga*, 845 F.2d 993, 998 (Fed.Cir.1988)). Thus, Noelle argues, the disclosure in his previous '799 application of the mouse form of CD40CR antibody was sufficient to support his later genus claims.

Noelle's reliance on *Staehelin* is misplaced. First, it is a decision from the Board of Patent Appeals and Interferences which may be persuasive but it is not binding precedent on this court. Second, the Board in *Staehelin* cited *Utter* to support the proposition that a patentee need not cite every species of an antibody in order to claim the genus of that antibody. In *Utter,* this court held that not every species of scroll compressor used in air conditioners must be described in order for a genus claim to meet the written description requirement. 845 F.2d at 994. Since the Board's decision in *Staehelin,* this court has subsequently held that a patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from species other than those specifically enumerated. *See Enzo Biochem II,* 323 F.3d at 965; *Regents,* 119 F.3d at 1568. Therefore, to the extent the Board's decision in *Staehelin* conflicts with our decisions in *Enzo Biochem II* and *Regents,* it has been limited in applicability.

The Board was also correct in its determination that the human and genus claims were anticipated by Lederman '771 and Armitage '974. The Board's decision was supported by substantial evidence, and Noelle conceded that without the earlier filing date of his '799 application, his claims were indistinguishable from the prior art cited by the Board.

B. *Interference-In-Fact*

[6] Interference proceedings are subjected to the requirements of 37 C.F.R. § § 1.601-1.690 (2003), promulgated pursuant to 35 U.S.C. § 135(a). *Eli Lilly v. Bd. of Regents of the Univ. of Wash.*, 334 F.3d 1264, 1267 (Fed.Cir.2003). A patent interference is designed to "determine whether two patent applications (or a patent application and an issued patent) are drawn to the same 'patentable invention' and, if so, which of the competing parties was first to invent the duplicative subject matter." *Id.* (citing *Conservolite, Inc. v. Widmayer*, 21 F.3d 1098, 1100-01 (Fed.Cir.1994)); *see also* 37 C.F.R § 1.601(j). [FN6] In order to determine whether the two parties claim the same patentable invention, the USPTO has promulgated a "two- way" test, which has been approved by this court. *Eli Lilly*, 334 F.3d at 1270. The two-way test reads as follows:

> ***7** Invention "A" is the same patentable invention as an invention "B" when invention "A" is the same as (35 U.S.C. 102) or is obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A". Invention "A" is a separate patentable invention with respect to invention "B" when invention "A" is new (35 U.S.C. 102) and non-obvious (35 U.S.C.

103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A".

37 C.F.R. § 1.601(n). In order for an interference-in-fact to exist, invention A must anticipate or make obvious invention B, *and* invention B must anticipate or make obvious invention A, thereby meeting both prongs of the "two-way" test. *Eli Lilly*, 334 F.3d at 1268; *accord Winter v. Fujita*, 53 U.S.P.Q.2d 1234, 1243 (Bd. Pat.App. & Int. Nov. 16, 1999). The Board in the present case worded the two-way test in a different way as follows:

> Thus, for Lederman to succeed in its motion for no interference-in-fact, Lederman need only demonstrate that: (i) Lederman's claims are not anticipated or rendered obvious by Noelle's remaining "mouse" claims; *or* (ii) Noelle's remaining "mouse" claims are not anticipated or rendered obvious by Lederman's claims.

(Emphasis in original).

[7] Noelle's argument that the Board improperly required a two-way patentability test, or, as the Board phrased it, a "one-way distinctiveness" test, is without merit in light of this court's recent ruling in *Eli Lilly* upholding the Director's two-way test as consistent with the language of the regulation. 334 F.3d at 1268. Therefore, the Board applied the proper "two- way test." First, it determined that "one skilled in the art lacked a reasonable expectation of success of obtaining Lederman's claimed 'human' subject matter when provided with Noelle's 'mouse' subject matter and using the screening techniques cited by Noelle." Although the Board did not have to conduct the second prong of the test to find no interference-in-fact, it did so anyway by finding that "one skilled in the art would have lacked a reasonable expectation of success of obtaining Noelle's 'mouse' subject matter when provided with Lederman's claimed 'human' subject matter and using the same screening methods." Therefore, the Board utilized the correct test to find no interference-in-fact.

Noelle's argument that the Board erred in its application of the obviousness question in the interference-in-fact analysis by ignoring the specification in Noelle's '480 application is also without merit. Both Lederman and Noelle concede that the anticipation portion of the interference-in-fact analysis is not an issue in light of the agreed variance between claims to mouse versus human forms of CD40CR antibodies. Thus, only the obviousness analysis pursuant to 35 U.S.C. § 103 is left to be determined. Obviousness is determined as follows:

> a proper analysis under § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success.

***8** *In re Vaeck*, 947 F.2d at 493. Both the suggestion and the reasonable expectation of success "must be founded in the prior art, not in the applicant's disclosure." *Id.; see also In re Dow Chem. Co.*, 837 F.2d 469, 473 (Fed.Cir.1988).

The parties agree that a skilled artisan would have been motivated to obtain the human CD40CR antibody if the mouse CD40CR antibody were available. The two parties disagree, however, as to whether the prior art would provide a reasonable likelihood of success in so doing. Therefore, the issue before us is whether substantial evidence supports the Board's determination that one of ordinary skill in the art would not have had a reasonable expectation of success of isolating the other party's invention given the disclosures found in the claims. A reasonable likelihood of success does not necessarily mean an absolute predictability, but rather a reasonable expectation of success. *Yamanouchi Pharm. v. Danbury Pharmacal, Inc.*, 231 F.3d 1339, 1343 (Fed.Cir.2000).

Noelle argues that the methods disclosed in his '799 patent application would have provided a reasonable likelihood of success for a person of ordinary skill in the art to isolate human CD40CR antibodies using mouse CD40CR antibodies. Specifically, Noelle argues it would have been obvious to a skilled artisan to use the CD40-Ig fusion protein disclosed in the '799 application as a screen to locate, within a hybridomal library, monoclonal antibodies that specifically bind to human CD40CR antigen. Noelle further argues the Board improperly ignored this method of antibody isolation merely because it was disclosed in Noelle's written description as opposed to Noelle's claims.

[8] The Board correctly found no interference-in-fact between Noelle's claims and Lederman's claims. First, the Board was correct in not considering Noelle's methods of isolation of human CD40CR antigen using CD40-Ig found in his '799 specification because the methods were neither part of the parties' inventions nor "prior art ." USPTO rules establish that an interference-in-fact exists when both parties claim the "same patentable invention." 37 C.F.R.

1.601(n). A patentee's invention is only found in a patentee's claims, unless the patentee uses sufficient means-plus-function language to invoke 35 U.S.C. § 112, paragraph (6). Thus, if the Board is to compare two inventions, the Board must only compare the parties' claims. Noelle does not claim a method of isolating CD40CR antigens, CD40-Ig, or the receptor CD40 itself. Obviously, if certain terms in Noelle's or Lederman's claims were ambiguous, we could resort to the specification or other sources to define those terms; however, it is unnecessary here as none of the terms in the claims are ambiguous. Therefore, Noelle cannot rely on a method of isolating human CD40CR antigen using CD40-Ig in order to prove obviousness between his invention and Lederman's invention because the method is not claimed.

*9 Second, the Board's determination was supported by substantial evidence because a person of ordinary skill in the art, given the state of prior art at the time of the '799 filing, would not have had a reasonable likelihood of success in isolating human CD40CR antibodies from the mouse CD40CR antigen and its antibody. Noelle argues that one skilled in the art would have had a reasonable likelihood of success in manufacturing a set of hybridomas that secrete monoclonal antibodies to activated human helper T-cell surface antigens. Noelle, as outlined previously, cited three different screening methods disclosed in his specification that would isolate the desired hybridomas and their antibodies. The first two of Noelle's proposed screening methods require the use of CD40-Ig. As the expert testimony of Dr. Aruffo, the named inventor in the patent claiming CD40-Ig, indicated to the Board, it would have been unpredictable and unreasonable to expect a skilled artisan to produce CD40-Ig given the state of the art at the time.

Finally, Noelle's expert witness, Dr. Clark, addressed the third and final proposed screening method. Dr. Clark declared that, given the mouse form of CD40CR antibody or CD40-Ig and the utilization of expression cloning methods available at the time, a person of ordinary skill in the art would have had a reasonable expectation of success in isolating the human form of CD40CR antigen. Armitage, however, during the prosecution of his '703 application, stated that the use of expression cloning could not have reasonably led to successful isolation of human CD40CR antigen.

After examining the record as a whole, we conclude there was substantial evidence to support the Board's decision. The Board's decision was reasonable in that, given the state of the art in the early 1990s as described by the expert witnesses, a person of ordinary skill in the art would not have had a reasonable likelihood of success in isolating human CD40CR antigen given mouse CD40CR antigen.

CONCLUSION

For the foregoing reasons, the decision of the Board rejecting claims 51, 52, 53, 56, 59, and 60 of Noelle's U.S. Application No. 08/742,480 is affirmed. The decision of the Board granting Lederman's preliminary motion of no interference-in-fact is also affirmed.

AFFIRMED

No costs.

FN1. For additional background on the function of antibodies, as well as methods of isolating antibodies, *see In re Wands*, 858 F.2d 731, 733- 34 (Fed.Cir.1988) and *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368-69 (Fed.Cir.1986).

FN2. Cell differentiation is the process of modifying a cell's structure and function in order for it to become more specialized and specific to the invading antigen.

FN3. CD40CR antigen is also referred to as "CD40 counter receptor," "CD40 ligand," "CD40L," and simply "CD40CR." Lederman uses the term "5c8 antigen" or "T-B cell-activating molecule" ("T-BAM") to designate the 30- kilodalton human form of CD40CR antigen. Noelle uses the term "gp39" (glycoprotein 39 kD) to describe the 39-kilodalton mouse form of CD40CR antigen.

FN4. A hybridoma is a man-made tissue culture consisting of cancerous B-cells fused to B-cells producing the antibody of choice. A hybridoma produces unlimited amounts of a desired "monoclonal" antibody. *See Hybritech*, 802 F.2d at 1368-69 (explaining the method for creating and using hybridomas).

FN5. CD40-Ig is a fusion protein wherein a portion of the CD40 receptor is fused to an immunoglobulin (Ig). CD40-Ig is therefore not expressed on the surface of a B-cell but rather is essentially a soluble, free-floating molecule.

FN6. 37 C.F.R. § 1.601(j) reads as follows: An interference-in-fact exists when at least one claim of a party that is designated to correspond to a count and at least one claim of an opponent that is designated to correspond to the count define the same patentable invention.

2004 WL 77931, 2004 WL 77931 (Fed.Cir.)

END OF DOCUMENT

United States Court of Customs and Patent Appeals.

Application of Alfred MARZOCCHI and Richard C. Horton.

Patent Appeal No. 8431.

April 15, 1971.

Appeal from decision of the patent office board of appeals which affirmed final rejection of claims 5, 6, 11 and 12 of application, serial No. 470,618, involving technique for improving adhesion characteristics between glass fibers and vinyl polymer resins. The Court of Customs and Patent Appeals, Baldwin, J., held that claims 5 and 11, teaching use of monomeric vinyl pyrrolidone, were obvious in light of reference teaching use of polymeric vinyl pyrrolidone, but that claims 6 and 12, reciting use of polyethyleneamine, were supported by disclosure which was in compliance with requirements of specification statute despite the breadth of the claim, where record contained insufficient grounds for questioning the accuracy of teaching that any polyethyleneamine would function to accomplish the asserted result.

Affirmed in part and reversed in part.

West Headnotes

[1] **Patents** 16.4
291k16.4 Most Cited Cases
(Formerly 291k18)

In connection with patent application involving technique for improving adhesion characteristics between glass fibers and vinyl polymer resins, claims 5 and 11, teaching use of monomeric vinyl pyrrolidone were obvious in light of reference teaching use of polymeric vinyl pyrrolidone. 35 U.S.C.A. § 103.

[2] **Patents** 16(3)
291k16(3) Most Cited Cases
(Formerly 291k18)

Inference of fact that, to one possessing the ordinary level of skill in the art, it would be obvious to try particular composition may at times be enough to justify drawing the ultimate conclusion of law that the claimed subject matter as a whole would have been obvious. 35 U.S.C.A. § 103.

[3] **Patents** 101(4)
291k101(4) Most Cited Cases

Claims 6 and 12 of patent application relating to technique for improving adhesion characteristics between glass fibers and vinyl polymer resins, which recited use of "polyethyleneamine" were supported by disclosure which was in compliance with specification statute despite contention as to excessive breadth of the disputed term, where record contained insufficient grounds for questioning accuracy of teaching that any polyethyleneamine would function to accomplish the asserted result. 35 U.S.C.A. § 112.

[4] **Patents** 101(4)
291k101(4) Most Cited Cases

Where generic term is recited in patent application, the only relevant concern of the patent office under specification statute should be the truth of the assertion that any member of the class will accomplish the desired result, not the breadth of the term. 35 U.S.C.A. § 112.

[5] **Patents** 101(4)
291k101(4) Most Cited Cases

Specification disclosure which contains teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the specification statute unless there is reason to doubt the objective truth of statements contained in the specification which must be relied on for enabling support. 35 U.S.C.A. § 112.

[6] **Patents** 113(7)
291k113(7) Most Cited Cases

Unpredictability of chemical reactions alone may be enough to create reasonable doubt as to accuracy of particular broad statement put forward as enabling support for claim, especially where the statement is, on its face, contrary to generally accepted scientific principles, but it is incumbent upon the patent office, when rejection is made on this basis, to explain why it doubts the truth or accuracy of the statement. 35 U.S.C.A. § 112.

[7] **Patents 101(4)**
291k101(4) Most Cited Cases

In considering accuracy of specification, pertinent references are not necessarily prior art references. 35 U.S.C.A. § 112.

Patents 328(2)
291k328(2) Most Cited Cases

2,853,465. Cited as reference.
****221 *1069** Herman Hersh, McDougall, Hersh, Scott & Ladd, Chicago, Ill., attorney of record, for appellant. Staelin & Overman, Toledo, Ohio, George A. Degnan, Arlington, Va., of counsel.

S. Wm. Cochran, Washington, D.C., for the Commissioner of Patents. Fred W. Sherling, Washington, D.C., of counsel.

***1070** Before RICH, ALMOND, BALDWIN and LANE, Judges, and DURFEE, Judge, United States Court of Claims, sitting by designation.

BALDWIN, Judge.

This is an appeal from the decision of the Patent Office Board of Appeals which affirmed the final rejection of claims 5 and 11 of appellants' application [FN1] under 35 U.S.C. § 103 as unpatentable in view of Werner [FN2] and of claims 6 and 12 under 35 U.S.C. § 112 as being based on an inadequate disclosure. Claims 4 and 10 stand allowed.

FN1. Serial No. 470,618, filed July 8, 1965, for 'Fiber Coatings-- Nitrogen Compounds for Improving Adhesion of Vinyl Polymers to Glass' as a continuation-in-part of Serial No. 96,106, filed March 16, 1961.

FN2. U.S. Patent No. 2,853,465, issued September 23, 1958.

THE INVENTION

The subject matter of the claims on appeal involves a technique for improving the adhesion characteristics between glass fibers and vinyl polymer resins. Claim 5 is representative and reads as follows:

5. In the combination of glass fibers and a vinyl polymer resin composition present as a coating on the glass fiber surfaces, the improvement which comprises mixing the vinyl polymer resin, prior to coating of the glass fibers, with an amine compound in an amount corresponding to 2-10% By weight of the vinyl polymer resin, and in which the amine compound is monomeric vinyl pyrrolidone.

Claim 11 is drawn to the same concept as claim 5, but defines the invention as 'a method of producing glass fibers coated with polyvinyl resin strongly bonded to the glass fiber surfaces.' Claims 6 and 12 differ from claims 5 and 11 respectively solely in the recitation of 'polyethyleneamine' as the critical 'amine compound' additive.

THE SECTION 103 REJECTION

Claims 5 and 11 were rejected 'as obvious in the sense of 35 USC 103 over Werner.' Werner, the sole reference relied upon here, is addressed to the improvement in the bonding relationship between glass and polyvinyl halide resins. The pertinent disclosure is as follows:

I have found that polyvinyl halide resins may be successfully modified so as to obtain excellent glass adhesion by employing a mixture of a polyvinyl halide and a polymer of N-vinyl pyrrolidone. By employing a mixture containing from 80 to 97% Of a polyvinyl halide and from 20 to 3% Of a polymer of N-vinyl pyrrolidone, which term includes homopolymers of vinyl pyrrolidone and copolymers with other polymerizable monomers, a composition is obtained having extremely high adhesion to all glass surfaces.

***1071** On the basis of this teaching the examiner took the position, accepted by the ****222** board, that the claimed use of monomeric vinyl pyrrolidone rather than Werner's polymeric vinyl pyrrolidone would be obvious to one of ordinary skill in the art since Werner's teaching would indicate to 'one skilled in the art * * * that it is the vinyl pyrrolidone moiety that is enhancing the adhesion.' It was also suggested by the examiner that since the claims recite no temperature conditions for the coating operation and since monomers polymerize when heated, the claims could possibly cover circumstances wherein the monomer is polymerized during application. The board appears to have accepted this suggestion and to

have extended it even further. It stated:

All of Werner's examples specify heating at elevated temperatures (110 degrees C.-130 degrees C., 165 degrees C., 325 degrees F., 350 degrees F) with and without elevated pressures. Appellants' specification says nothing about retaining the vinyl pyrrolidone in monomeric form, much less anything about 'maximizing adhesion' by preventing polymerization. Indeed, the very designation of the vinyl pyrrolidone as a 'monomeric' material introduced into a polymer system for the purpose of altering the properties of such system implies subsequent polymerization of the monomer. Appellants' further argument that the monomer has entirely different capabilities and solubilities than the polymer is also unpersuasive.

Appellants' position on appeal in response to these assertions by the examiner and board is largely to stress again the 'marked difference between the properties and characteristics of a polymer as compared to a monomer,' and to object to the 'purely conjectural' assertion that the monomer polymerizes in the coating after it is applied. Additionally, appellants make the following contention:

Even if it were assumed that appellants' monomeric vinyl pyrrolidone is polymerized when present in the polyvinyl chloride coating, there is no teaching or suggestion in Werner that the use of monomeric vinyl pyrrolidone has any efficacy whatsoever in compositions of the type disclosed and claimed. The basis suggested by the Patent Office for the rejection is tantamount to the allegation it would be 'obvious to try' the monomer. This 'test' of obviousness has been frequently repudiated by this court.

[1] The sole issue is, of course, whether the Werner teaching does suggest to a person having ordinary skill in this art that the use of monomeric vinyl pyrrolidone would have the efficacy indicated in the appealed claims. We agree with appellants that whether the monomer polymerizes is irrelevant, at least in this regard. What is relevant, however, and here determinative, is the examiner's assertion that the Werner teaching would suggest that it is the vinyl pyrrolidone moiety alone and not some other characteristic peculiar to a polymer which is efficacious in producing the desired adhesion enhancement. ***1072** [FN3] In the absence of anything to rebut this assertion, which is reasonable on its face, we are constrained to accept it as fact. The inferences which follow from such fact, i.e., that the monomer would possess this same characteristic and that one of ordinary skill would recognize such fact, are inescapable.

> FN3. Indeed, the reasonableness of such an assertion is confirmed by the very disclosure contained in appellants' application which indicates that efficacious adhesion enhancers are those 'organic nitrogenous compounds which are characterized both by an organic constitution which is compatible with the vinyl polymers and by a polarity expressed in the nitrogen function.' As also pointed out by appellants in their brief (about which more will be said later), the nature of the present invention resides in the use of amine compounds, broadly, as adhesion enhancers.

[2] It is acknowledged that the above line of reasoning may be viewed as being tantamount to drawing the inference that, to one possessing the ordinary level of skill in this art, it would be 'obvious ****223** to try' the monomer. Nevertheless, such an inference of fact may, at times, be enough to justify drawing the ultimate conclusion of law that the claimed subject matter as a whole would have been obvious under section 103. We are satisfied that the circumstances of this case justify an initial conclusion of obviousness. Since the record before us contains nothing to rebut that conclusion, the decision with regard to claims 5 and 11 must be affirmed.

THE SECTION 112 REJECTION

Claims 6 and 12, which recite the use of 'polyethyleneamine' as the adhesion enhancer, were criticized by the examiner as being based on a disclosure which was not enabling under the first paragraph of 35 U.S.C. § 112. The board affirmed his rejection of those claims with the following comment.

The term is obviously generic to a considerable number of compounds varying in the number of ethylene groups, the number of amine groups and the relationship of the polyethylene groups to the amine groups, and accordingly does not provide a reasonable guide for those seeking to improve the adherence of vinyl resins to glass.

[3] We will reverse the board's decision on this rejection since we are unable to find sufficient justification for the holding that appellants' disclosure is not enabling.

[4] Turning specifically to the objections noted by the board as indicated above, it appears that these comments indicate nothing more than a concern over the breadth of the disputed term. If we are correct, then the relevance of this concern escapes us. It has never been contended that appellants, when they included the disputed term in their specification, intended only to indicate a single compound. Accepting, therefore, that the term is a generic one, its recitation must be taken as an assertion by appellants that all of the 'considerable ***1073** number of compounds' which are included within the generic term would, as a class, be operative to produce the asserted enhancement of adhesion characteristics. The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion. The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.

[5] As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt does exist, a rejection for failure to teach how to make and/or use will be proper on that basis; such a rejection can be overcome by suitable proofs indicating that the teaching contained in the specification is truly enabling.

[6][7] In the field of chemistry generally, there may be times when the wellknown unpredictability of chemical reactions will alone be enough to create a reasonable doubt as to the accuracy of a particular broad statement put forward as enabling support for a claim. This will especially be the case where the statement is, on its face, contrary to generally accepted scientific principles. Most often, additional factors, such as the teachings in pertinent references, [FN4] will be available to substantiate any doubts that the asserted scope of objective enablement ****224** is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof. In any event, it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure. Cf. In re Gazave, 379 F.2d 973, 54 CCPA 1524 (1967); In re Chilowsky, 229 F.2d 457, 43 CCPA 775 (1956).

> FN4. Not necessarily prior art references, it should be noted, since the question would be regarding the accuracy of a statement in the specification, not whether that statement had been made before.

In the present case, the circumstances we see do not support the reasonableness of any doubts which the Patent Office might have had ***1074** concerning the adequacy of appellants' specification disclosure to support these claims. In fact, those circumstances tend to strengthen rather than weaken appellants' claim to the breadth of protection they seek. In the first place, it has not been asserted by the Patent Office that the chemical properties of known polyethyleneamines vary to such an extent that it would not be expected by one of ordinary skill in this art that any such compound would possess the necessary capability of enhancing adhesion. Additionally, we note that polyethyleneamine is listed in appellants' specification as being only one of a much larger class of amine compounds possessing this necessary characteristic. Finally, we recognize (as did the examiner) the generic nature of appellants' broader concept, i.e., that the desired property of adhesion enhancement stems largely from the amine moiety. It does appear that variation of certain of the secondary factors mentioned by the examiner, such as molecular weight or proportion of ethylene groups, might influence to some degree or even mask the essential 'amine' property of the polyethyleneamine or its obviously equally essential compatibility with vinyl polymers. However, we see no basis to conclude that the ready avoidance of this result would not be within the level of ordinary skill in this art. Compare In re Skrivan, 427 F.2d 801, 57 CCPA 1201 (1970).

Taking all these circumstances into consideration, we are constrained to conclude that the record before us contains insufficient grounds for questioning the accuracy of appellants' teaching that any polyethyleneamine (obviously excepting those whose essential 'amine' characteristics and compatibility

with vinyl polymers would be masked by the secondary factors mentioned) will function to accomplish the asserted result. It follows that claims 6 and 12 must be held to be supported by a disclosure which is in compliance with the requirements of the first paragraph of 35 U.S.C. § 112.

SUMMARY

The decision of the board regarding claims 5 and 11 is affirmed; that dealing with claims 6 and 12 is reversed.

***1069** Modified.

439 F.2d 220, 58 C.C.P.A. 1069, 169 U.S.P.Q. 367

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